ECRONICON OPEN ACCESS

EC GYNAECOLOGY
Research Article

A Comparative Study: Bioavailability and Effect of Organic and Inorganic Zinc Compounds on Male Reproductive System

Oksana Shtapenko[1]*, Alla Madich[2], Vasyl Syrvatka[3], Oksana Slyvchuk[1] and Yevhen Dzen[4]

[1]*Laboratory of Reproductive Biotechnology, Institute of Animal Biology NAAS, Ukraine*

[2]*Department of Genetics, University of Cambridge, United Kingdom*

[3]*Department of Genetic and Biotechnology, Ivan Franko National University of Lviv, Ukraine*

[4]*Laboratory of Metabolism Named After Stepan Gzhytskiy, Institute of Animal Biology NAAS, Ukraine*

***Corresponding Author:** Oksana Shtapenko, Laboratory of Reproductive Biotechnology, Institute of Animal Biology NAAS, Lviv, Ukraine.

Received: January 06, 2023; **Published:** February 20, 2023

Abstract

The study was aimed to compare bioavailability and effect of organic and inorganic Zn compounds on male reproductive system development in mice as experimental models. Zinc glutamic acid chelate and zinc sulphate heptohydrate compounds were administered by intraperitoneal route in inbred males, 0.5 mg Zn per kg of body weight, twice per week, for 35 days. The animals of control group received ddH_2O. The males were sacrificed on day 14^{th}, 28^{th} and 40^{th} given that whole cycle of germ cell generation takes 8.6 days in mice. Counting, motility, morphology and density of epididymis sperm cells were accepted as a main proof of Zn compounds bioavailability. Morphological changes in seminiferous tubules and germinal epithelium were evaluated histologically by hematoxylin/eosin staining. The mice receiving Zn-Glu showed substantial increases in testes and seminal vesicle weights, extensive histomorphological changes in testicular tissue followed by improved spermatogenesis and increased number of motile and vital sperm cells that was the best indicator of epididymis secretory activity in comparison with other groups. Basic seminal parameters in $ZnSO_4$ group were not elevated. Research data confirmed a bioavailability of Zn glutamic acid chelate and most beneficial effect of Zn-Glu in mice, while $ZnSO_4$ could have a detrimental effect.

Keywords: *Reproduction; Zinc Glutamate; Bioavailability; Sperm; Motility*

Introduction

Participation Zinc (Zn) in important biochemical and physiological processes is associated with the activity of over 300 enzymes [1,2] that makes Zn indispensable structural, catalytic, intercellular and intercellular signal component for such cell functions as gene expression, DNA and protein synthesis [3,4], signal transduction, replication, cell signaling, cell division also [5,6]. About 10% of all proteins in mammalian body can be bound by Zn [7] that acts as a catalytic and structural co-factor in a few hundred of metalloenzymes, particularly the DNA-binding proteins with Zn fingers [8], membrane protein that regulates apoptosis [9] and even proteins that plays role of mechanotransducers for endothelial and vascular smooth muscle cells [10] for proper functioning of body systems.

$Zn(^{2+})$ and fertility is a key point for many discussions because some statements suggest Zn deficiency correlates with a decrease in male fertility but they contradict other studies claiming there is no significant association between Zn and sperm quality.

There are lots of evidence $Zn(^{2+})$ linked with key events in the acquisition of fertilization ability by spermatozoa, including sperm motility, capacitation, membrane stabilization and acrosome reaction [11,13,14]. Zn homeostasis is highly regulated in the prostate, because $Zn(^{2+})$ plays an important role in sperm release and motility [12]. It has also been show a positive correlation between $Zn(^{2+})$ concentration and development of testes, prostate, sperm concentration and capacitation as well as the good morphology of spermal cells [15,16]. There is a well-known fact, Zn associates with sperm cells protection against oxidative stress being the co-factor of superoxide dismutase (Cu/Zn-SOD) [17]. The absence or moderate deficiency of Zn in the seminal plasma leads to oxidative damage risk increasing, low sperm quality and idiopathic male infertility [18]. $Zn(^{2+})$ reduction may result the germ cell death without the formation of mature sperm cells for meiosis period and lead to the changes in the structure, proliferation and differentiation of sperm cells that is the evidence of its regulatory activity in spermatogonial proliferation [19].

Zn is virtually supplemented in all animal diets, but mostly as inorganic trace mineral salts likely sulphates, chlorides, and oxides. However, in spite of the cheap cost, the use of inorganic Zn compounds is characterized by limited uptake because does not provide the body with amount of the mineral required for male fertility. It is believed that $Zn(^{2+})$ present in the body in organic form mainly. Furthermore, organic Zn compounds are slowly released within the cell, thus cells have enough time to regulate the free $Zn(^{2+})$ customizing after disintegration of organic compounds within cell cytoplasm [20].

Lots of experimental studies carried out to investigate the influence of organic Zn compounds on different organs and tissue in animals, but nothing is known about Zn glutamic amino acid chelate long-term exposure effects on male reproductive system (including bioavailability), especially when Zn-Glu is administered by intraperitoneal route. To date, a few forms of chelated mineral supplements have been developed for animal and poultry diets, and potential advantages of an organic Zn over an inorganic compound have been confirmed [21]. For instant, it has been reported that supplementation of Zn-methionine amino acid complex (Zn-Meth) improved growth performance, nutrient utilization and mineral metabolism in male lambs and higher bioavailability of Zn propionate organic compound was confirmed [22]. Relative bioavailability of chelated Zn to compare with Zn sulfate or Zn oxide was higher in chicken`s diets [23]. However, these data were not confirmed by others [24]. Meta-analysis data indicated that the form of supplemental Zn was not a limiting factor for dietary Zn bioavailability in broilers and piglets [25]. Therefore, such inconsistency of bioavailability of different organic mineral compounds can depend from the nature of ligand and strength of chelation, solubility and stability of Zn compounds.

In order to investigate Zn organic compound bioavailability, Zn^{2+} chelate of glutamic amino acid has been employed in this study. We considered that the use of glutamic acid as a soluble organic product can enhance the effect of Zn amino acid chelate on male reproductive system because of the involvement of glutamate as neurotransmitter in the activation of hypothalamic-pituitary axis. Thus, the main objective of the comparative study was to investigate the effect of Zn glutamic amino acid chelate (Zn-Glu) versus inorganic Zn sulfate heptahydrate ($ZnSO_4$), as an alternative source of $Zn(^{2+})$, on male reproductive system and understanding the processes of sperm maturation in mice as experimental model for effect evaluation.

Materials and Methods

Animals and experimental design

Inbred C57BL/6J mice 8 - 10 weeks old males, weight 27 - 30g, were used in this experimental study. The animals were housed under standard laboratory conditions, such as 12h light: 12h dark cycle, relative humidity of 50 ± 5% and room temperature 22 ± 3°C. They were maintained on pelleted diet and water Ad-libitum, in social groups, 5 animals per cage.

Zn glutamate (Zn-Glu) - Zinc glutamic acid chelate $C_5H_7NO_4Zn$- is organic complex of Zn $(^{2+})$ with L-glutamic acid and potentially a water molecule.

Zn sulphate ($ZnSO_4$) - heptahydrate $ZnSO_4 \times 7H_2O$ - is the inorganic $Zn(^{2+})$ compound.

The mice males were divided randomly into three groups, 30 animals per group, and initial body weights have been recorded. The animals of the experimental group 1 (EG-1) received intraperitoneal injection of Zn-Glu, and the experimental group 2 (EG-2) received $ZnSO_4$ solution at a dose 0.5 mg Zn per kg of body weight, twice per week, for 35 days. The treatment duration was concerned according to the features of spermatogenesis in mice given that the overall differentiation from spermatogonia cells to mature spermatozoa requires 35 days [26]. Experiment was conducted over 9 treatments by intraperitoneal route. The males of the control group were administered with double distilled water (ddH_20) on same days.

Spermatogenesis is tightly orchestrated process within seminiferous tubules periodically cycling through 12 epithelial stages and defined by the combination of germ cells present. The completion of one cycle takes 8.6 days in mice [27]. In our study, on day 14^{th}, 28^{th} and day 40^{th} since the Zn compounds first administration 10 males in each group were sacrificed by cervical dislocation. Thus, we assumed that two (8.6 days x 2 = 17.2 days), three and four generations of germ cells were presented within the seminiferous tubules on the sacrificing days including a short time for adaptation (for 5 days since the last treatment) the solubilized Zn compounds to the body system after last drug administration.

The reproductive organs were removed from the sacrificed animals, separated from each other for weight recording and tissue analysis to reveal Zn compounds possible effect.

Reproductive organ collection

The testes with cauda epididymis and seminal vesicles were removed and weighted after excluding of the adipose tissue and blood vessels on the surface of the epididymis. The organs were observed on asymmetry between the right and left sides. The gonadosomatic index (GSI) was calculated as the rate of the testis weight (g) to the body weight (g) (%): GSI = Testis weight (g)/Body weight (g).

Epididymis sperm count, sperm motility and vitality assessment

A cauda epididymis tissue was cut off at the caudal end at right or left side in each male and used for sperm collection and counting. Sperm motility and vitality were assessed according to the WHO Laboratory Manual (2021) [28]. The cauda epididymis tissue from each male was placed in a 35-mm plastic culture dish separately (NUNCLON, Nalge Nunc Inter Co., Japan), then minced with a surgical scalpel thoroughly and homogenized in sterile phosphate-buffered saline solution (PBS, pH=7.2) at 37°C. Tissue fragments were subsequently removed, and sperm suspensions collected for incubation for 10 min at 37°C. The sperm suspension from each epididymis was diluted in dH_2O 1:4 then and mixed thoroughly to prevent sperm movement. Sperm concentration was determined by total motile counts in each sample under a light-contrast microscope and multiplied by 5×10^6 to calculate the number of sperms per epididymis.

Semen quality was examined as the percentage of motile and vital cells after incubation under a phase-contrast microscope (Olympus Co., Tokyo, Japan, x400). One hundred spermatozoa per animal was evaluated and classified into four motility categories: rapid progressively motile (+), slow progressively motile (±), non-progressive (-) and immotile (0). The number (%) of vital, morphologically abnormal, and immature sperms was assessed in an equal volume of semen samples that mixed with 0.5% eosin and evaluated under magnification x400.

Histological studies

The testes were harvested post-mortem on day 14^{th}, 28^{th} and 40^{th} of treatment, 10 animals per group, fixed in 10% formaldehyde, dehydrated, and embedded in paraffin according to the standard histological technique. Serial 4 μm-thickness paraffin sections have been made by tissue microdisectioning followed by hematoxylin/eosin staining and observed under magnification x400.

Statistical analysis

The measurement data were presented as the mean ± standard deviation (SD) of at least three individual experiments. One-way analysis of variance (ANOVA) was performed using the Statistical Analysis Systems (Origin 9.0) software package. The statistical significance of P values was determined by the Bonferony's test at the same significance level. The level of significance was set at $P < 0.05$.

Results

Organ weight

The outcomes of organic/inorganic Zn and male reproductive system interactions were different, depending on bioavailability of the Zn compounds. This relations reflected in the absolute weights of testes, cauda epididymis tissue and seminal vesicles in animals treated and sacrificed on day 14th, 28th and 40th. There was no difference between left and right testes in animals but the enhanced effect of Zn-Glu in EG-1 group was significantly higher when compared with $ZnSO_4$-treated males in EG-2 group and control animals (ddH2O). Statistically significant increases in testes and seminal vesicle weights were seen in mice administered with Zn glutamic acid chelate on days 28th and 40th ($P < 0.05$) (Figure 1A and 1B). Increases in testes and seminal vesicle weights in Zn-Glu-treated males were combined with other responses.

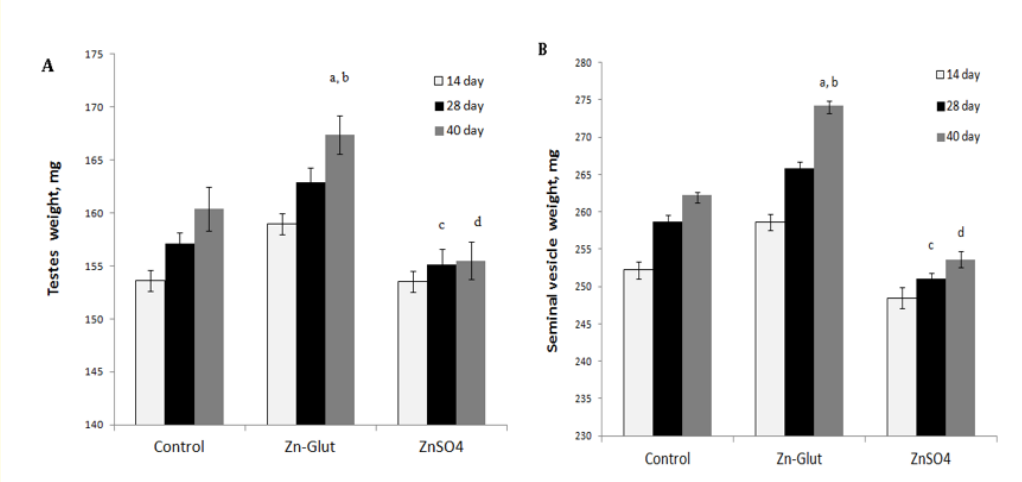

Figure 1: *Testes (1A) and seminal vesicle (1B) weights in males administered with Zn-Glu or $ZnSO_4$ and sacrificed on days 14th, 28th and 40th of treatment. Data are expressed as means ± SD for 10 mice, $P < 0.05$. Significance compared: [a]Control to Zn-Glu groups on day 40th; [b]Zn-Glu group on day 14th to 40th; [c]Zn-Glu to $ZnSO_4$ groups on day 28th, [d]Zn-Glu to $ZnSO_4$ groups on day 40th.*

Relatively low testes and seminal vesicles weights were recorded in males administered with Zn sulfate (EG-2 group) even in comparison with control animals. Markable decreases in seminal vesicle weights that occurred in EG-2 males already on the two-week regimen under Zn sulfate influence could be associated with some degenerative changes there.

Slightly increased epididymis weight was observed in mice of all groups on 40th days of treatment. There were no significant differences of between groups of animals sacrificed on day 14th and 28th but probability of essential difference between animals sacrificed on days 14th and 40th has been recorded in each group that can be related to the animal growing ($P < 0.05$, Figure 2).

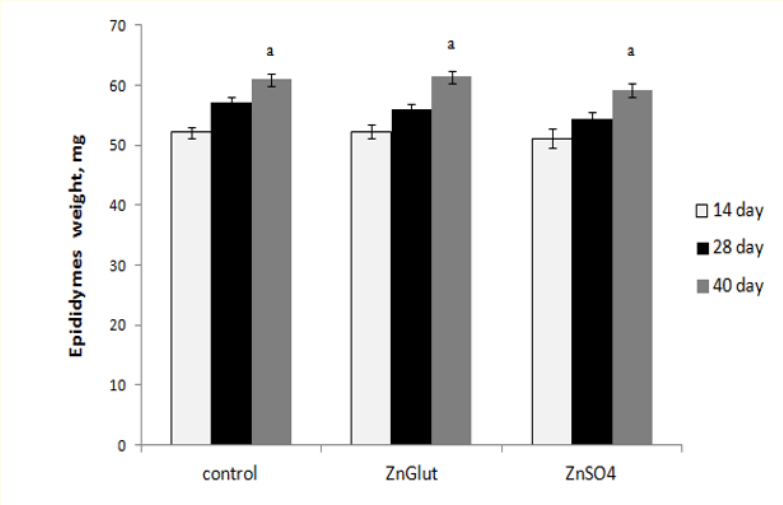

Figure 2: *Epididymis weights in males administered with Zn-Glu or $ZnSO_4$ and sacrificed on day 14^{th}, 28^{th} and 40^{th} of treatment. Data are expressed as means ± SD for 10 mice, P < 0.05. Significance compared: [a]Day 14^{th} to 40^{th} in each group.*

Because testes are less dependent on body weight than seminal vesicles, which are presented adjusted for body weight, testes weight was taken as additional criteria for the Zn compounds effect assessment. Administration of organic and inorganic Zn compounds induced the changes in male reproductive organs and reflected in Gonadosomatic index (GSI) that correlates with testes weight (Figure 3).

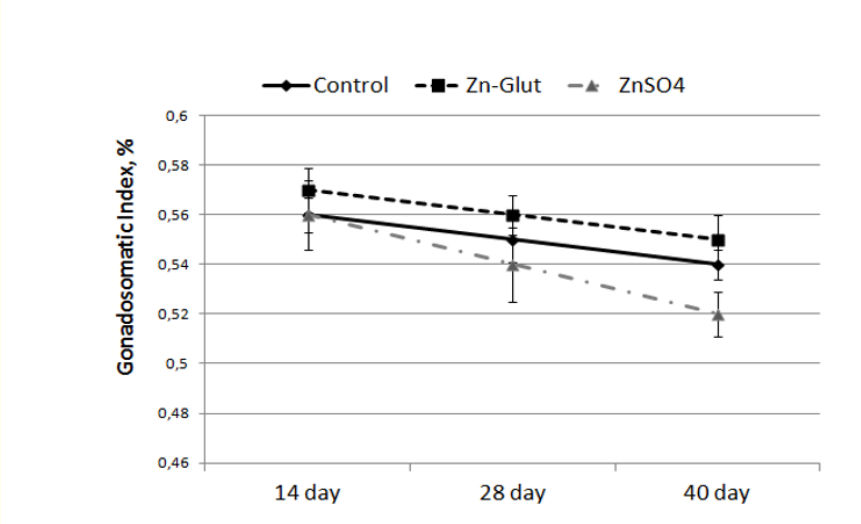

Figure 3: *Gonadosomatic index of Zn-Glu- and $ZnSO_4$-treated males sacrificed on day 14^{th}, 28^{th} and 40^{th} in comparison with control animals.*

The highest GSI(s) were found in Zn-Glu chelate-treated group, while the lowest rate was observed in mice administered with $ZnSO_4$ that indicates lower bioavailability of inorganic Zn. It should be underscored that the described changes in male reproductive system function in mice under influence of the water-soluble Zn compounds involved cell/tissue interactions that are tightly related to sperm count, motility and vitality regulation and were extensively discussed in the following subsections.

Epididymal sperm parameters

By post-mortem examination of epididymis sperm cell suspensions some changes between the males in different groups were detected throughout the study period. The results of sperm counting as well as sperm motility and vitality analysis showed that the organic and inorganic water-soluble Zn compounds did affect to epididymal sperm parameters in different way (Figure 4).

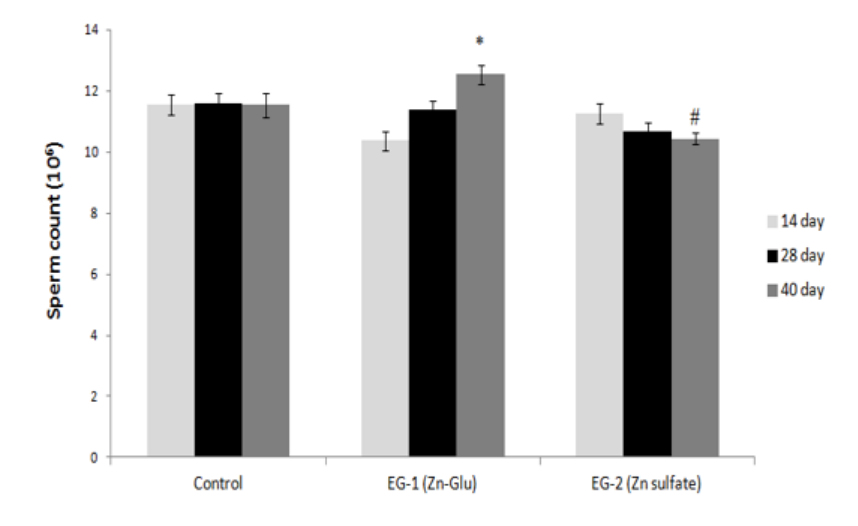

Figure 4: *Sperm counting in samples from males administered with Zn-Glu or $ZnSO_4$ on day 14th, 28th and 40th of treatment. Data are expressed as means ± SD for 10 mice, P < 0.05. Significance compared: *In Zn-Glu group and #In $ZnSO_4$ group on day 40th.*

The number of sperm cells in samples from Zn-Glu-treated animals (EG-1 group) increased significantly in timing manner towards day 40th (P < 0.05). In animals administered with inorganic Zn compound (EG-2) the sperm cells concentration decreased gradually during the observation period (P < 0.05) that can be concerned as epididymis function inhibition under Zn sulfate influence.

Our finding revealed a significant effect of Zn-Glu administration to the sperm motility and vitality. Only the number of cells recorded as sperm cells with rapid progressive motility were calculated as a key index of sperm quality. The results of these studies are summarized in table 1 and globally address an enhanced activity of seminiferous tubules of testes and epithelium function they are lined with.

Motility is regarded as normal if the number of rapid progressive sperm cell > 25% or rapid and slowly progressive sperm cell number > 50%. Significant differences in sperm motility were recorded between groups in this study. The sperm cells characterized as progressively motile being raised gradually in Zn-Glu group for all observation time. The highest number of motile sperm and the lowest percentage of immotile sperm were observed in samples from animals treated with organic Zn compound on day 40th, 78.1% in comparison with 65.9% in control (P < 0.05) and 55.9% in inorganic Zn compound group (P < 0.01).

Duration of treatment, days	Rapid progressive motile (%)	Slow progressive motile (%)	Non-progressive motile (%)	Immotile (%)	Sperm motility (%)
Control group, dd$H_2$0					
14	45,5 ± 1,64	14,5 ± 1,19	19,5 ± 0,73	19,8 ± 1,87	60.0
28	49,8 ± 1,18	14,4 ± 1,33	18 ± 0,93	18,3 ± 1,32	64.2
40	51,1 ± 0,89	14,8 ± 0,92	15,4 ± 0,6	18,7 ± 0,93	65.9
Zn-Glu -treated (EG-1 group)					
14	50,1 ± 1,35	14,2 ± 0,89	18,1 ± 0,72	17,7 ± 0,96	64.3
28	54,3 ± 1,7	17 ± 1,11	14,8 ± 0,65	13,9 ± 1,22	71.3
40	59 ± 1,37[a]	19,1 ± 1,0	12,2 ± 0,68	9,7 ± 1,03[c]	78.1
$ZnSO_4$-treated (EG-2 group)					
14	43 ± 1,94	13,3 ± 1,38	20,8 ± 0,94	22,8 ± 2,45	56.3
28	39,4 ± 1,89	19,6 ± 1,36	17,7 ± 0,62	23,3 ± 2,32	59.0
40	38,1 ± 2,58[b]	17,8 ± 1,22	19,1 ± 0,97[b]	24,7 ± 1,89[bd]	55.9

Table 1: *Sperm motility on day 14th, 28th and 40th of treatment in males administered with Zn-Glu or $ZnSO_4$.*

*Legend: Data are expressed as means ± SD for 10 mice, P<0.05. Significance compared: [a]control to Zn-Glu group on day 40th; [b]Zn-Glu to $ZnSO_4$group on day 40th; [c]in Zn-Glu group on day 40th; [d a] control to $ZnSO_4$group on day 40th; **Sperm motility (%) is number of sperm cells categorized as Rapid and Slow progressive motile cells together.*

On the contrary, the animals that were treated with inorganic Zn compound displayed a significant reduction of the progressively motile sperm number and characterized with highest number of immotile sperm on day 14th, 28th and 40th in comparison with control and EG-1 groups.

The percentage of vital sperm cells were assessed by counting 100 sperm cells per animal, 30 samples in each group, based on normal and abnormal morphology. Abnormal morphology was regarded when a sperm head was smaller than normal, the neck was broken, degenerated tail shape marked. Morphological observation showed there were significant changes of sperm vitality in males of EG-1 group as an effect of Zn-Glu administration for first 14 days. The extended administration of Zn-Glu gave further improvement. Particularly, sperm morphology significantly increased in EG-1 group on day 40th to compare to day 14th ($P < 0.05$).

The lowest number of vital sperm were seen in sperm suspensions from animals treated with inorganic Zn compound and sacrificed on day 40th in comparison with control and EG-1 groups ($P < 0.05$). The results of epididymis sperm vitality are described in figure 5.

Histological examination of tissues

The testicular structure was evaluated by the cellular makeup assessment of the morphological changes in seminiferous tubules and epithelium they are lined up. The photomicrographs in figure 6 illustrate the histopathologic changes in testes samples of mice sacrificed on day 14th, 28th and 40th of treatment.

Testicular sections from control males didn't show any histopathology changes in seminiferous tubules that displayed active spermatogenesis. There were no significant differences in control and Zn-Glu treated groups of animals when sacrificed on day 14th (Figure

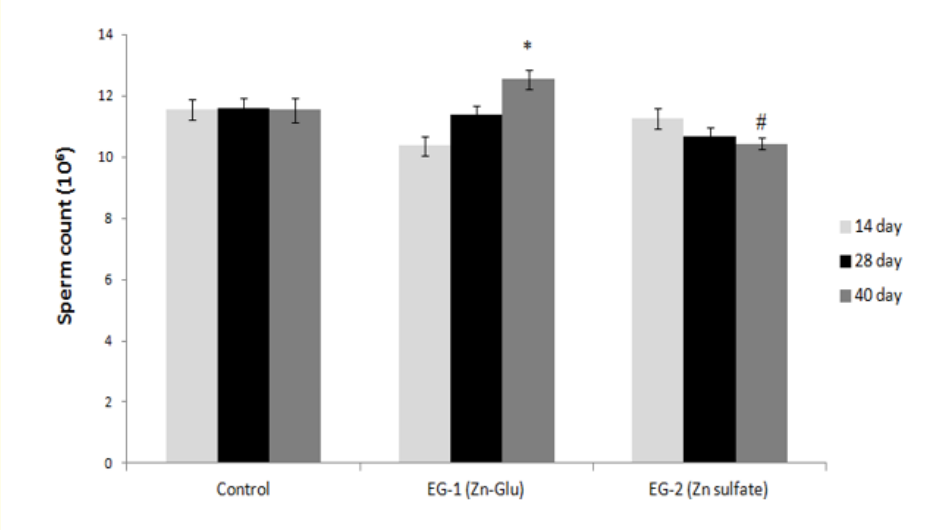

Figure 5: *Sperm vitality in males treated with Zn-Glu or $ZnSO_4$ and sacrificed on day 14^{th}, 28^{th} and 40^{th}. Data are expressed as means ± SD for 10 animals per group, P < 0.05. Significance compared: [a]Control to Zn-Glu groups on day 40^{th}; [b]Control to $ZnSO_4$ groups on day 40^{th}; [c]Zn-Glu to $ZnSO_4$ groups on day 40^{th}.*

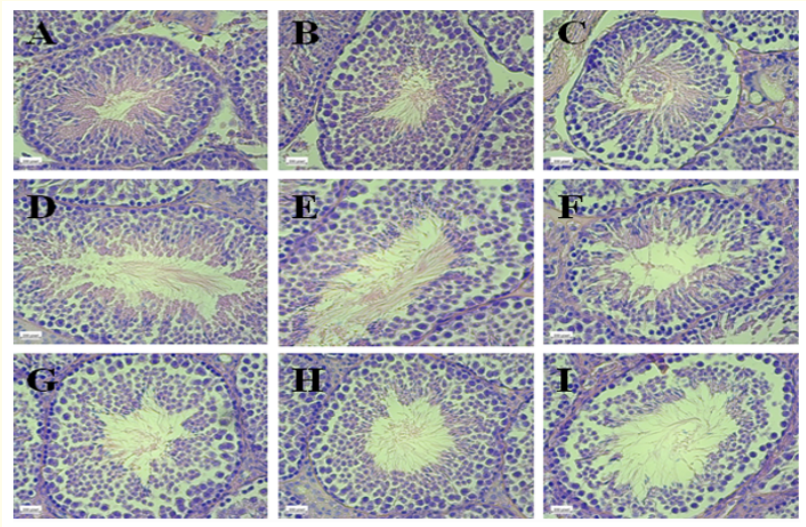

Figure 6: *Histopathological photomicrographs of testes tissue in mice: control group (A, D, G); animals of EG-1 group treated with 0.50 mg Zn/kg BW as Zn-Glu (B,E,H) and animals of EG-2 group treated with 0.50 mg/kg Zn/kg BW as $ZnSO_4$ (C, F, I) on day 14^{th} (A-C), 28^{th} (D-F) and 40^{th} (G-I) following treatment (HE staining; ×100; scale bar, 200 µm).*

6A and 6B). Both groups exhibited a compact and regular arrangement of cells in the seminiferous tubules (Figure 6A, and 6B respectively). However, the seminiferous epithelium was tighter in the control group than in EG-1, and the number of spermatogonia, primary spermatocytes and Leydig cells visibility was better in the control group in comparison with Zn-Glu group, while as the samples in Zn-Glu group characterized with slightly wider lumen. In fact, such changes could lead to further development of epithelial tissue, shorter time for sperm production, and speedier changes of the spermatogenic cell phenotype towards to matured sperm cells.

While the control animals demonstrated testicular tissue with thickness and cell appearance as normal, the samples from mice in Zn-Glu treatment group have had well-developed interstitial tissue in the seminiferous tubules lined with numerous Sertoli cells on day 28^{th} and 40^{th}. Additionally, the increased number of matured sperm cells as cells with large nuclear and higher density of younger sperm cells such as elongated spermatids in the lumen were easy visible (Figure 6B, 6E and 6H).

Unlikely the control and Zn-Glu groups, an exposure of males on $ZnSO_4$ for 14 days caused some testicular lesions, such prominent damages as the exfoliation of germ cells into the lumen of seminiferous epithelium, increased cell distance and deceased cell layer even (Figure 6C, at the upper left corner). The number of Sertoli cells that are usually located at the inner membrane of a seminiferous tubules was dramatically decreased in EG-2 group samples. Long-term treatment with $ZnSO_4$ might induce a reduction of sperm cell number, vacuolization, impairment of cell motility and abnormal cell morphology.

More significant differences have been observed in $ZnSO_4$-treated group of animals sacrificed on day 28^{th} and 40^{th}. The seminiferous tubules were situated at larger cell distances, they had some germ cell layer disturbance and low number of elongated spermatids in the lumen, as well as significant vacuolization of the Sertoli cells (Figure 6F, 6I). There was a separation of germinal epithelium in tubules that says about possible tissue necrosis affected by disturbance in common regulation, especially spermatogenesis.

Discussion

This is a first report that focuses on the effect of the Zn glutamic acid chelate on male reproductive system in mice, semen quality and morphological changes in seminiferous tubules and epithelium in testes tissue. The present study demonstrated that Zn-Glu chelate, in comparison with $ZnSO_4$ heptohydrate as a counterpart and alternative source of inorganic $Zn^{(2+)}$, was more bioavailable and had a positive impact on the growth of spermatogonia improving semen quality in 12 - 16 weeks old males. The significant differences of organic and inorganic Zn compounds effect on male reproductive system was demonstrated. Several markers have been used to evaluate the impact, such as reproductive organ weights, testicular tissue histomorphology, sperm density, motility and vitality.

Animals that were treated with Zn-Glu chelate displayed significant growth of testes and seminal vesicles. It was not reach in $ZnSO_4$-treated group that is the indicator of better bioavailability of the chelated Zn. The slightly bigger epididymis weight in first experimental group EG-1 was detected at the last-check procedure only, on day 40^{th}, and this phenomenon remains unclear because cauda epididymis is organ that less dependent from body weight than seminal vesicles.

The reduction of testicular weights in Zn sulphate-treated animals might correlate with low bioavailability of inorganic Zn compound in this study and can be associated with the zinc-deficiency [29]. The earlier study revealed that Zn-deficient diet can cause reduction of testes size up to 42% in rats [30]. In our investigation the intraperitoneal injection of $ZnSO_4$ for 35 days led to the seminal vesicles growth repress for whole time of observation. Since this gland is responsible for secretion of nutrients, specifically such modulators of sperm motility as seminal plasma motility inhibitor, seminal vesicle autoantigen and PATE [31], there was a possibility that the lower number of progressive motile sperm cells in $ZnSO_4$-treated animals could be related with impaired seminal secretion.

The direct action of the Zn on the spermatogenesis is well-known. $Zn^{(2+)}$ plays a significant role in early sperm cell growing, germ cells meiosis, spermatogonial reproduction and fertility [32]. Mostly, Zn assembles in germ cells and an overall increasing of intracel-

lular Zn concentration improves germinal cell proliferation [33]. Zn deficiency leads to a depletion of total germ cell mass via impaired spermatogenesis and increased apoptosis of the testicular cell sub-population. The germ cells are highly adapted to take up $Zn^{(2+)}$ at each developmental stage. Particularly, epididymis spermatozoa are capable to rapidly load extracellular Zn from luminal cavity during their transit through the epididymis [34]. That is why Zn bioavailability means so much.

The present study showed that the Zn-Glu administration increased the sperm density in the samples and improved sperm motility and sperm morphology to compare with control animals. The data are in accordance with other earlier research on Zn supplementation in different species. It was revealed that Zn supplementation significantly increases the sperm ejaculate volume, sperm motility and number of sperm cells with good morphology in men [35]. However, some authors did not find a positive correlation between Zn level and some sperm quality parameters. Despite of some divergences, all authors agreed that Zn impacts positively on sperm cell growing and motility. There is an opinion, the mechanisms by which Zn might influence on the sperm function is its ability to maintain the stability of sperm chromatin and membrane stabilization. It is essential to keep an optimal level of incorporation of Zn into sperm chromatin because it contributes to the compact chromosomes to fit in the sperm cell nucleus that is leading to chromatin condensation [36]. During spermatogenesis, Zn acts as the regulator of disulphide cross-links SH-Zn-SH between protamine macromolecules in the tightly condensed chromatin [32]. Testicular spermatozoa are immature and get motility and fertilization ability during transit, protection and storage in the epididymis [37]. During transit throughout the epididymis (caput, corpus, and cauda), spermatozoa interact with the highly specialized luminal microenvironment, where the secretory activity of the tubular epithelium is forming, thanks to epididymiosomes (extracellular microvesicles enriched with cholesterol and proteins), miRNA, and other macromolecules [38]. In current study, sperm production, sperm motility and vitality were increased as an outcome of Zn glutamic acid chelate in EG-1 males and slightly but statistically significant decreased in animals administered with Zn sulfate in comparison to the control group. The finding suggests, Zn-Glu affects the reproductive performance in males through extensive histomorphological changes in cauda epididymis followed by increasing spermatogenesis, or followed by inducing oxidative stress and possible testicular toxicity in mice subjected to non-bioavailable compound treatment.

Microscopic observation of testicular tissue was focused on the effect of chelated Zn-Glu on sperm cells generation on different stages of spermatogenesis that located in the base of seminiferous tubules. Most commonly, improvement of the histological properties and distinctive increase of the sperm cells in lumen compared to the control paraffin sections were in male treated with Zn-Glu. Conversely, the frequency of testes sections containing different histopathological changes including germinal epithelium damage showed that Zn sulphate administration decreased the number of pachytene spermatocytes on day 14^{th} that was reflected on the epididymis sperm parameters of this group. We can assume that damage of Sertoli cell junctions might implicate the separation of the germinal epithelium which affected the development of germ cells and could led to abnormal sperm development. This finding could be attributed to the disordered arrangement in this cells, so that lowering cell number was compensated by the increasing distance between cells.

Conclusion

In the current study some difference effects of organic and inorganic zinc compounds on male reproductive performance in mice were revealed. It can be concluded that zinc glutamic acid chelate causes significant increasing in testis and seminal vesicle weights, improves sperm count, motility and vitality as well as holds the activity of germinal epithelium function in testicular tissue. The results suggest that Zn-Glu is more effective than $ZnSO_4$ in terms of improved structural integration in the reproductive organs, shows better bioavailability and indicates the beneficial effect on epididymis sperm parameters while $ZnSO_4$ could have a detrimental effect. Use chelated Zn-Glu in dose 0.5 mg Zn per kg of body weight at long-term exposure by intraperitoneal drug administration can be recommended for male reproductive function improvement and replace inorganic form of Zn.

Conflict of Interest

The authors report no conflict of interest.

Bibliography

1. Vickram S., *et al*. "Role of Zinc (Zn) in Human Reproduction: A Journey from Initial Spermatogenesis to Childbirth". *International Journal of Molecular Sciences* 22.4 (2021): 2188.

2. Cheah Y and Yang W. "Functions of essential nutrition for high quality spermatogenesis". *Advances in Bioscience and Biotechnology* 2.4 (2011): 182-197.

3. Senkiv OM., *et al*. "Activites of enzymes antioxidant defence in tissues of piglets for actions of different level of zinc". *Bìologìâ Tvarin* 16.1 (2014): 133-139.

4. Kumar N and Singh AK. "Role of Zinc in Male Infertility: Review of Literature". *Indian Journal of Obstetrics and Gynecology Research* 3.2 (2016): 167-171.

5. Cousins RJ., *et al*. "Mammalian zinc transport, trafficking, and signals". *Journal of Biological Chemistry* 281.34 (2006): 24085-24089.

6. Kluska K., *et al*. "Metal binding properties, stability and reactivity of zinc fingers". *Metallomics* 10.2 (2018): 248-263.

7. Andreini C and Bertini I. "A bioinformatics view of zinc enzymes". *Journal of Inorganic Biochemistry* 111 (2012): 150-156.

8. Cassandri M., *et al*. "Zinc-finger proteins in health and disease". *Cell Death Discovery* 3 (2017): 17071.

9. Miyamoto K., *et al*. "The zinc finger domain of RING finger protein 141 reveals a unique RING fold". *Protein Science* 26.8 (2017): 1681-1686.

10. Siddiqui MQ., *et al*. "Bioinformatic Analysis of Structure and Function of LIM Domains of Human Zyxin Family Proteins". *International Journal of Molecular Sciences* 22.5 (2021): 2647.

11. Vickram AS., *et al*. "Human prostasomes an extracellular vesicle–Biomarkers for male infertility and prostate cancer: The journey from identification to current knowledge". *International Journal of Biological Macromolecules* 146 (2020): 946-958.

12. Agarwal A., *et al*. "Proteomics, oxidative stress and male infertility". *Reproductive BioMedicine Online* 29.1 (2014): 32-58.

13. Fallah A., *et al*. "Zinc is an essential element for male fertility: A Review of Zn roles in men's health, germination, sperm quality, and fertilization". *Journal of Reproduction and Infertility* 19.2 (2018): 69-81.

14. Allouche-Fitoussi D and Breitbart H. "The role of zinc in male fertility". *International Journal of Molecular Sciences* 21.20 (2020): 7796.

15. Mirnamniha M., *et al*. "An overview on role of some trace elements in human reproductive health, sperm function and fertilization process". *Reviews on Environmental Health* 34 (2019): 339-348.

16. Dissanayake DMAB., *et al*. "Relationship between seminal plasma zinc and semen quality in a subfertile population". *Journal of Human Reproductive Sciences* 3.3 (2010): 124-128.

17. Garratt M., *et al*. "Copper-zinc superoxide dismutase deficiency impairs sperm motility and in vivo fertility". *Reproduction* 146.4 (2013): 297-304.

18. Colagar AH., *et al*. "Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men". *Nutrition Research* 29.2 (2009): 82-88.

19. Khan MS., *et al*. "Assessment of the level of trace element zinc in seminal plasma of males and evaluation of its role in male infertility". *International Journal of Applied and Basic Medical Research* 1.2 (2011): 93-96.

20. Maares M and Haase H. "A guide to human zinc absorption: general overview and recent advances of in vitro intestinal models". *Nutrients* 12.3 (2020): 762.

21. Hara T., *et al*. "Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis". *The Journal of Physiological Sciences* 67.2 (2017): 283-301.

22. Brooks M., *et al*. "Bioavailability in chicks of zinc from zinc propionate". *The Journal of Applied Poultry Research* 22.2 (2013): 153-159.

23. Sahraei M., *et al*. "Estimation of the relative bioavailability of several zinc sources for broilers fed a conventional corn-soybean meal diet". *The Journal of Poultry Science* 50.1 (2013): 53-59.

24. Zafar MH and Fatima M. "Efficiency Comparison of Organic and Inorganic Minerals in Poultry Nutrition: A Review". *PSM Veterinary Research* 3.2 (2018): 53-59.

25. Schlegel P., *et al*. "Bioavailability of zinc sources and their interaction with phytates in broilers and piglets". *Animal* 7.1 (2013): 47-59.

26. DeLouise LA. "Applications of nanotechnology in dermatology". *Journal of Investigative Dermatology* 132.3-2 (2012): 964-975.

27. Ernst C., *et al*. "Staged development mapping and X chromosome transcriptional dynamics during mouse spermatogenesis". *Nature Communications* 10 (2019): 1251.

28. WHO laboratory manual for the examination and processing of human semen, Sixth Edition (2021): 276.

29. Chen Y., *et al*. "Zinc deficiency promotes testicular cell apoptosis in mice". *Biological Trace Element Research* 195.1 (2020): 142-149.

30. Omu AE., *et al*. "Molecular basis for the effects of zinc deficiency on spermatogenesis: An experimental study in the Sprague-dawley rat model". *Indian Journal of Urology* 31.1 (2015): 57-64.

31. Noda T and Ikawa M. "Physiological function of seminal vesicle secretions on male fecundity". *Reproductive Medicine and Biology* 18 (2019): 241-246.

32. Foresta C., *et al*. "Role of zinc trafficking in male fertility: from germ to sperm". *Human Reproduction* 29.6 (2014): 1134-1145.

33. Yamaguchi S., *et al*. "Zinc is an essential trace element for spermatogenesis". *Proceedings of the National Academy of Sciences of the United States of America* 106.26 (2009): 10859-10864.

34. Franca LR., *et al*. "Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs". *Theriogenology* 63.2 (2005): 300-318.

35. Zhao J., *et al*. "Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis". *Scientific Reports* 6 (2016): 22386.

36. Björndahl L and Kvist U. "A model for the importance of zinc in the dynamics of human sperm chromatin stabilization after ejaculation in relation to sperm DNA vulnerability". *Systems Biology in Reproductive Medicine* 57.1-2 (2011): 86-92.

37. James ER., *et al*. "The role of the epididymis and the contribution of epididymosomes to mammalian reproduction". *International Journal of Molecular Sciences* 21.15 (2020): 5377.

38. Marchiani S., *et al*. "Epididymal sperm transport and fertilization". In: Simoni M., Huhtaniemi I. (editions) Endocrinology of the Testis and Male Reproduction. Endocrinology. Springer, Cham (2022).

Volume 12 Issue 3 March 2023