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Research Article

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The effects of zinc supplement on fertility in male mice

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ABSTRACT

This study was carried out to investigate the effects of zinc supplement in organic and mineral forms on spermatogenesis and fertility of mice. Seventy male mature mice in seven groups (control and 50,100, and 150 mg/kg of each form) with ten members in each group were studied in a 35 days period. At the end of period, the amount of seminiferous stem cells, primary spermatocytes, histological changes of epididymis slides, the amount of epididymal sperm and relative testis weight were measured. Fertility was also studied. Obtained data were analyzed using one way ANOVA and Duncan's test using SPSS program. According to results, testicular histology showed that testis weight was increased in 50 and 100 organic groups. The amount of stem cells and primary spermatocytes were increased significantly in 50 and 150 organic groups and all mineral groups. The amount of epididymal sperm and fertility were reduced significantly in all treatment groups ($p<0.05$). In general, zinc supplement can reduce reproduction potential of mice dose dependently by affecting proliferation of spermatogonia.

Key words: Zinc supplement, Fertility, Mice

INTRODUCTION

Zinc is a rare mineral which has the second amount in body after iron. It is stored mainly in muscles but can be found also in red and white blood cells, retina, bones, kidneys, liver, and pancreas [1].

Zinc strengthens the immunity and protects skeleton growth, feather production and skin. Furthermore, using zinc supplements causes increase in weight and conversion ratio of broilers. This element is a rare necessary element for all live systems including bacteria, plants, animals and human. The importance of zinc was declared after 1934. It was known as parakeratotic reason of pigs' skin injuries, slow growth, weak feather production, and abnormal skeleton growth of birds. Recent papers about birds propose that zinc deficiency in poultry is related to low quality of corpse and foot injuries due to scratches and damage to the skin. This led to poultry breeders and nutritionists to believe that zinc supplements are vital for birds [2].

Zinc plays key role in immune system improvement and activity of some sexual hormones. Deficiency of this element can have destructive effects on epithelial tissue of chickens' reproduction systems. It also affects uterine discharges and reproduction system, so that affects the duration of membrane shell synthesis in the isthmus, Lime shell in the womb, and saving albumin in magnum [3].

Carbonic anhydrase enzyme was known first at 1940 by Clinvman. This enzyme has about 0.33% of zinc. Various researches have shown that adding zinc supplements to diet improves the activity of this enzyme and reduced Shell defects. This enzyme combines H_2O and CO_2 and makes H_2CO_3 which finally produces carbonate anions (Co_3) in endometrial cells. This anion in combined with $Ca_2$and produces calcium carbonate which is main compound of shell [4].

This study was carried out to investigate the effect of zinc supplement on fertility of male mice.

EXPERIMENTAL SECTION

The study was conducted in research laboratory of Islamic Azad University, Isfahan Branch. Seventy male mice in weight range of 30±5g from Balb/C race were kept for 14 days to adapt to environment. Samples had free access to food and water, 25-30$^{o^C}$ temperature and natural light. During this period samples had free access to normal light, water and pellet diet. Noise was also in minimal level. The duration of the study was 35 days. Mice were divided in seven groups including control and 50,100, and 150 mg/kg of each form.

Zinc supplements were powders of glycine zinc and mineral zinc with 40% and 90% purities which were added to drink water. At the end of period testes plus epididymis were separated, their fats were removed and were weighed using digital weigh (0.01g).

Formalin fixation solution was used to prevent tissue changes or injuries. Sperms were counted using Neobar lam and proparing testes slides were done using H&E coloring method.

In day 35, five mice of each group were selected and were kept in separate cages with two female mice. Sixteen days later, embryo numbers in uterine branches were counted and compared with control group to determine the fertility amount.

Obtained data were analyzed using SPSS program and one way ANOVA and means were compared using Duncan multiple ranges test at 5% probability level.

RESULTS AND DISCUSSION

Testis weight was significantly affected by treatments ($p<0.05$) and was increased in 50, and 100 mg/kg organic and 50 and 150 mineral groups (Figure 1).

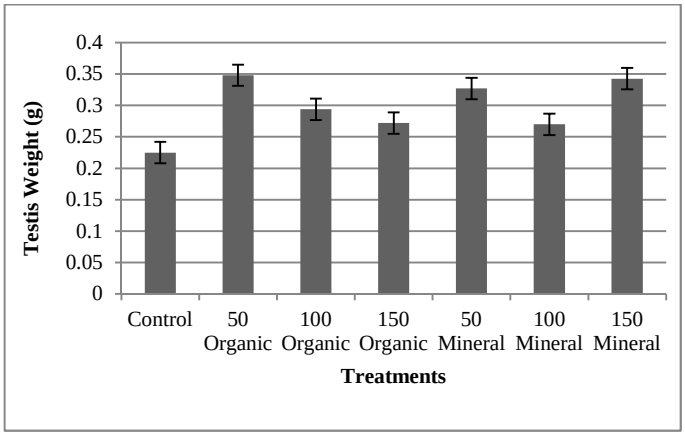

Figure 1. Testis weight in all groups

The number of sperm stem cells was increased significantly ($p<0.05$) in all groups except 100 organic group (Figure 2).

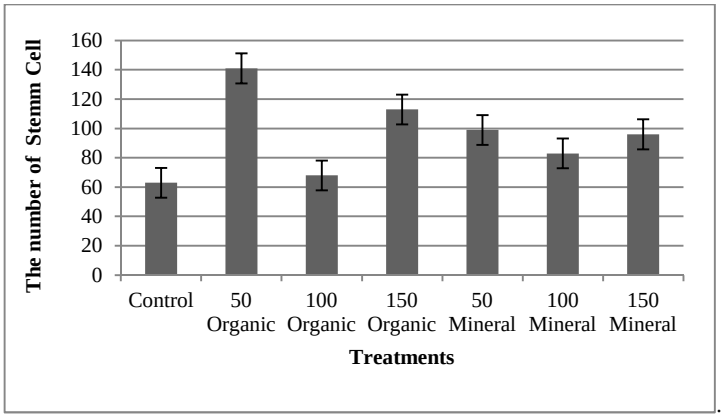

Figure 2. The number of sperm stem cells in all groups

The number of primary spermatocytes was significantly increased in all groups except 100mg organic group (Figure 3).

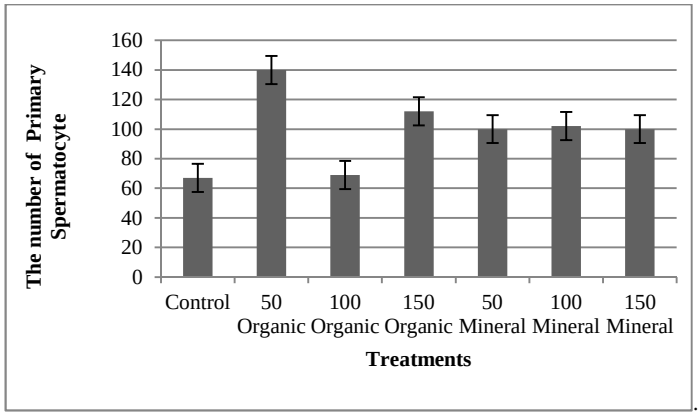

Figure 3. The number of primary spermatocytes in all groups

Zinc supplement had significant effect on epididymal sperm amount and reduced it in all groups. The amount of epididymal sperm was very low in 150 mg organic group (Figure 4).

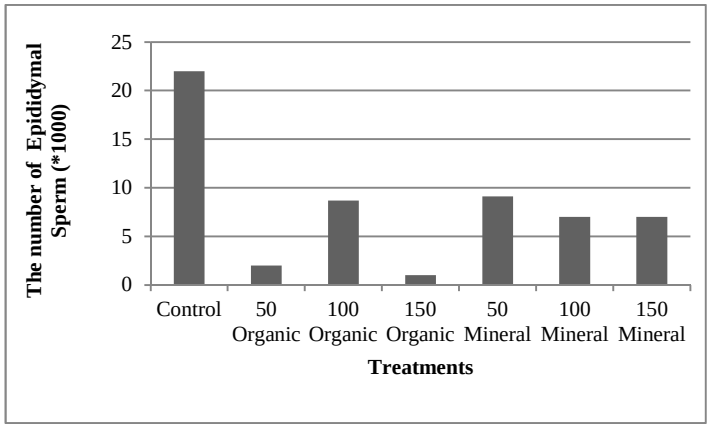

Figure 4. The number of epididymal sperm amount in all groups

Fertility amount was significantly reduced by zinc in all groups and was very low in 150 mg organic group (Figure 5).

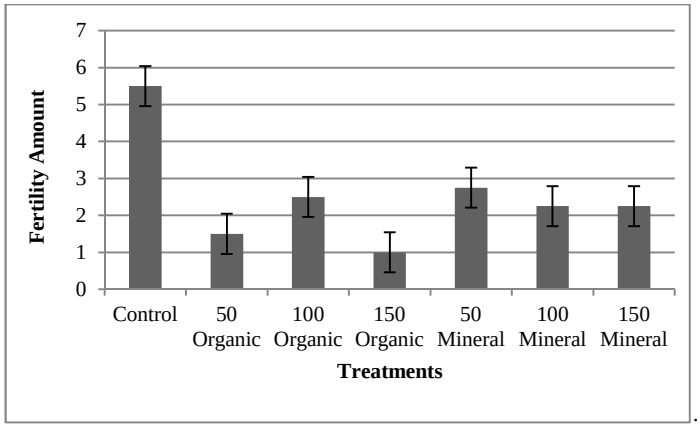

Figure 5. The fertility amount in all groups

The amount of testis stem cells and primary spermatocytes were increased in all experimental groups except 100mg organic group significantly which shows dose dependent effect of zinc. Previous studies have shown also effective role of zinc in cellular system and mitosis increase in different tissues including testis. This is not only related to mitosis but also gonadotropins and testicular hormones were affected by different doses. Since FSH effect is essential for seminiferous tubes to produce spermatozoid [5]. Study of supplements effects on FSH showed that zinc especially mineral form by 90% purity could increase the FSH amount. FSH causes growth of seminiferous tubules and controls the action of sertoli cells in testes [1].

Zinc has direct effect on maturity of sperm cells and preserving the germinal epithelium of male sex glands. It also plays role in growth and development of male sexual organs and their natural activity. Zinc deficiency cause delay in growth and development of testes and prohibits spermatogenesis [6].Zinc supplements are effective in reproduction cycle of various species and are essential for spermatogenesis synthesis [2].The effects of zinc on prostate gland are very obvious. Zinc deficiency reduces testosterone discharge and prevents spermatogenesis. Spermatozoids failures have been observed in rats with zinc deficiency which is in agreement with our results [7].

Relative testis weight was increased by supplements. In general, weight and size of testis are related to its performance directly. Therefore, increase in its weight has positive effects on spermatogenesis activity and production of androgen hormone in testis and testosterone increment increases testes weight [8]. Increase in testes weight in this study can be ascribed to increases in the amounts of stem cells and primary spermatocytes. Lydig cells make androgen hormones which are transferred to blood and Sertoli cells. Part of testosterone which is reached to Sertoli cells is converted to estradiol by aromatase enzyme. Zinc deficiency in male goats caused smaller testes and decreased libido [9]. Lack many of the nutrients affect fertility indirectly. Effects of these nutrients are enforced via effecting metabolism. The most obvious sign of zinc deficiency is smaller gonads [7].

As it is seen in microscopic slides of epididymis, zinc supplement has reduced the amount of epididymal sperm significantly. Sperm making which includes complicated accurate cell differentiation processes in mammals is started in maturity and continued along reproduction time: stem cells are divided and the result of meiosis divisions are haploid spermatids which are processed totally in testes and epididymis to make a complete sperm. All spermatogenesis stages are done simultaneously and even a small interruption can cause infertility [8]. Zinc supplements interrupted sperm making process via direct effect on epididymis spermatozoids and apoptosis. Researches show that the concentration of mineral zinc is in related to ALP. ALP was increased in all mineral groups. Higher amounts of enzyme may occur slowly and Temporary but shows liver inflammation or injuries [1].

Zinc deficiency in bulls affected sperm production and delayed sperm maturity [4]. Both organic and inorganic zinc supplementation in the diet improved seminal characteristics of the bulls. Zinc supplement can destroy somniferous tubes and lydigo hepatocytes of liver [6]. Increase in concentration of blood's zinc causes destruction and necrosis of sexual organs, liver and kidney [7].

Zinc deficiency in bulls affects sperm production. It Reduced sperm production, delayed sperm maturity, and reduced growth of calves. This is not in agreement with results of current study.

Results of this study showed that fertility amount was decreased in next generation by zinc supplement, which is due to intensive reduction in epididymal sperms in most of treatment groups. Since certain number of sperm in the semen is necessary for fertilization, this reduction will directly affect the number of fertilized ovules of female mice

and can be a strong reason for dose dependent effect of zinc on reproductive potential reduction [1]. Zinc affects reproduction cycle of various species and is essential for spermatogenesis synthesis, maturity of spermatogenesis, ovulation and fertility [9].

According to results zinc in both forms can effect spermatogenesis process and reduce male sexual cells intensively.

CONCLUSION

It seems also that this reduction is effective on hypothalamus- pituitary- gonadal axis but it depends on zinc form. Thus, it can be announced that zinc have negative effect on reproduction processes and sexual potential of male sex.

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