

EFFECT OF LIPOSOMAL PREPARATE WITH SOME ORGANIC TRACE ELEMENTS ON ANTIOXIDANT STATUS AND REPRODUCTIVE ABILITY OF FEMALE RABBITS

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Abstract

The paper aimed to investigate the influence of subcutaneous injections of organic microelements in liposomal forms on the performance antioxidant status in blood of female rabbits and their reproduction function during the early stage of pregnancy. Two weeks before fertilization females from experimental group were subcutaneous injected preparations with Zn glutamate, Mn glutamate, Cr methionine, NaSe with vitamins E, A, D in liposomal form. Reproductive organs and blood samples were obtained after hormonal induction and fertilization on 14th day of gestation. Our results showed that supplemental organic trace elements increased the number of implantations and corpus luteum in the ovaries in the experimental group as compared with the control group. The activity of catalase has decreased in the experimental group compared with control group. However, the increasing ceruloplasmin activity in experimental group was noted. Liposomal preparation injection induced the significantly decrease of content oxidatively modified proteins (OMP) in the blood of the experimental group ($p < 0.05$; $p < 0.01$) compare to that index in control group. Accordingly, TBARS level in experimental group significantly ($p < 0.01$) decreased, as compared with the control. The results of our study indicate that supplementation organic microelements in liposomal form before 2 weeks and during fertilization has a positive effect on the reproductive ability of female rabbits to improve the pregnancy, implantation rates. Addition of organic microelements in liposomal form provided increase of antioxidant defense system and lower intensity of peroxidation.

Key words: organic trace elements, rabbits, reproduction.

INTRODUCTION

Organic trace minerals make more of the trace mineral bioavailable to the animal than inorganic trace minerals. Numerous studies in cattle, rabbits and poultry have shown improvements in reproductive performance, immune system function and mineral status when complexed organic trace minerals were used. Bioavailability of organic Zn, Cu, Mn and Se relative to inorganic salts has been evaluated in many studies (Pavlata et al., 2012; Cao et al., 2000). Zinc functions as a catalytic, structural and signaling factor in the regulation of a diverse array of cellular pathways involving hundreds of enzymes and proteins. Zinc is an important factor necessary for regulating the meiotic cell cycle and ovulation (Kim A.M., 2011). Many studies have shown that zinc deficiency before

conception causes fertility and pregnancy problems, abnormal embryo and fetal development. The study by Tian X. and Diaz F. (2013) noted that zinc deficiency decreases histone and DNA methylation in oocytes. Manganese is an essential element utilized by antioxidants, including superoxidedismutase (MnSOD), and others metalloenzymes that take part in reduction reactions, in multiple physiological processes including reproductive system (Kim S. I., 2012).

Added to that, the combination of trace minerals used in the present study can help assure delivery of the essential trace minerals that can affect reproduction, including implantations rates, embryo development during the early stage of pregnancy.

The objective of this study was to evaluate the effect of subcutaneous injections of organic microelements in liposomal form for 2 weeks prior to mating on implantations and on

antioxidant status in blood of female rabbits during the early stage of pregnancy.

MATERIALS AND METHODS

The study was conducted on female rabbits divided into two groups: experimental and control. Female rabbits experimental group were subcutaneous injection 5 ml liposomal preparation with Zn glutamate (35 µg/kg), Mn glutamate (32 µg/kg), Cr methionine (60 µg/kg), NaSe (20 µg/kg) with vitamins E, A, D two weeks before fertilization.

Artificial insemination with appropriate hormonally treatment was performed in all group of animals. We used 40 IU PMSG (Follimag, Intervet, Holland) for synchronized cycle (was injected 48 h before AI) and 20 µg/doe GnRH (Gonadotropin-releasing hormone) (Fertagil, Intervet, Holland) for induction of ovulation (was injected at the moment of insemination). Rabbits were fertilized intravaginally of 10×10^6 spermatozoa/doe in 0.5 ml tris-citrate diluents. Rabbits were slaughtered on day 14th of gestation. The weight of ovarian and uterine, number of implantations and corpus luteum, the indices of fertilization and pregnancy were determined.

The blood samples were collected for determining antioxidant enzyme activities and levels of lipid peroxides. Activities of antioxidant enzymes such as catalase, ceruloplasmin, and, as well as oxidative stress biomarkers (thiobarbituric acid reactive substances (TBARS) and stable 2,4-dinitrophenyl hydrazine derivates of the oxidative modified carbonyl groups level) were measured.

RESULTS AND DISCUSSIONS

The effects of liposomal preparation on reproductive parameters are summarized in table 1. The mean number of corpus lutea in experimental group and the control group were 11.8 ± 0.74 and 10.8 ± 1.02 , respectively. Data analysis showed that the number of corpus lutea in the experimental group increased in compare with the control group. The injection of liposomal preparation with some organic trace elements showed positive

effect on the female in number of implanted embryos. However, the number of resorption in the female rabbits treated with liposomal preparation and control group were in similar level. While the pre- and post-implantation losses were lower in experimental group liposomal-treatment animals than the control (Table 1).

Table 1. Effect of liposome preparation on reproductive ability of female rabbits

Parameters	Control	Experimental
Number of corpora lutea	10.8±1.02	11.8±0.74
Number of implantation sites	9.8±1.52	10.8±0.74
Total Live Fetuses	9.2±1.36	10.2±1.14
Number of resorption sites	0.4±0.24	0.4±0.24
Pre-implantation losses (%)	11.1	10.2
Post-implantation loss (%)	4.2	3.7
Total gestational losses	14.8	13.5

Values are given as mean ± SD for 5 rabbits in each group.

Our results agree with those obtained by Diaz Francisco J. et al., 2014. They used zinc prior to ovulation, and it had marked positive effects on the mice's fertility. The study by Alikwe P.C.N. et al., 2011, showed that dietary supplementation of rabbits with zinc was carried out to determine its effects on reproduction performance and growth rate of rabbits.

We have studied the influence of liposome preparation with some organic trace elements on antioxidant status and lipid peroxidation in the female rabbits during the early stages of pregnancy. The obtained data showed that catalase activity decreased compared with control values (Figure 1). However, the increasing ceruloplasmin activity in experimental group was noted (Figure 2).

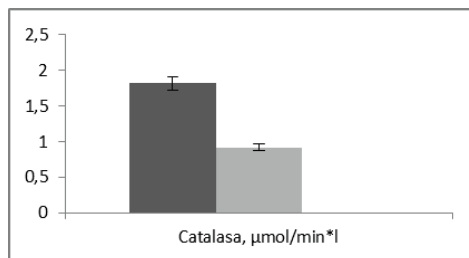


Figure 1. Effect of subcutaneous injections of organic microelements in liposomal form on catalase activity in female rabbits

Values are expressed as mean ± SD for 5 rabbits in each group.

Catalase is one of the important antioxidant enzymes regulating the levels of intracellular hydrogen peroxide and hydroxyl radical. It is known that the trace elements as Zn, Mn, Cu, Se are involved in the metabolic activities via metalloenzymes (Cu-Zn SOD, Mn SOD, Catalase, GSH-Px, etc.), which are essential for the antioxidant protection of cells (Ozturk-Urek R. et al., 2001).

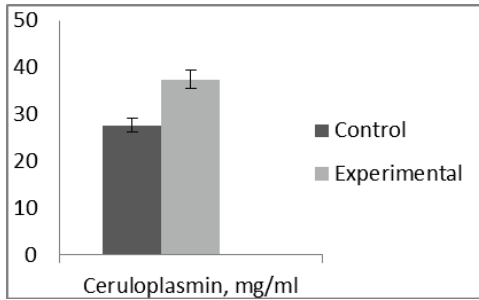


Figure 2. Effect of subcutaneous injections of organic microelements in liposomal form on ceruloplasmin activity in female rabbits.

Values are expressed as mean \pm SD for 5 rabbits in each group.

In our study the decreasing of catalase activity in experimental female rabbits which might be having been due to depletion or inhibition of the enzyme was found. As a result of decreasing of catalase activity production of free radicals increased during early gestation period, but serum ceruloplasmin activity in experimental group increased as compared to the control group.

Figure 3 show the effect of administration of liposomal preparation with some organic trace elements on serum lipid peroxidation and oxidative modification of proteins. Thiobarbituric acid reactive species (TBARS) level significantly decreased ($p < 0.01$) up 1.76 ± 0.06 , when compared with 2.64 ± 0.06 in the control value (Figure 3). The observed decrease of TBARS concentration in co-treated group is agreement with investigations Oshiro M., 2001, and can be explained by the enhanced activities of SOD. In many papers it have described the correlation between plasma concentration of the level of trace elements and SOD activity of erythrocytes, because copper, zinc and magnesium are the main components of SOD that plays a vital

role as an antioxidant and protects from oxidative stress.

Intensification of free radicals oxidation leads to oxidative modification of proteins (OMP), destruction of nucleic acids, sugars, and causes to structural and metabolic damages in the cells. Initiation of OMP is the most dangerous link in the cell damages, which leads to cytoplasmic enzymes and membrane ion pumps inactivation with gradual initiation different mechanisms of cell apoptosis.

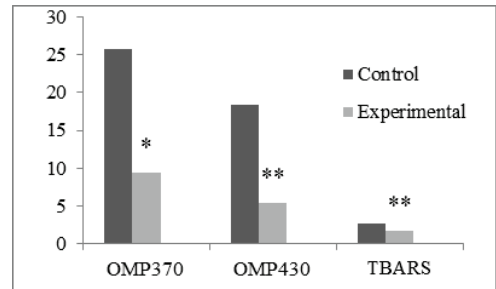


Figure 3. Effect of subcutaneous injections of organic microelements in liposomal form on OMP content and TBARS level in female rabbits

Oxidatively modified proteins (OMP) content, measured by quantity of carbonyl oxidation (aldehyde derivatives, OMP₃₇₀; ketonic derivatives, OMP₄₃₀) in the blood of females rabbits from control (non-treatment) and liposomal preparation injected experimental groups.

Values are expressed as mean \pm SD for 5 rabbits in each group. Figures in parenthesis are differences relative to control Significantly different from control * - ($p < 0.05$); ** - ($p < 0.01$).

However, destruction of proteins is a more reliable marker of oxidative damages in tissues than the products of lipid peroxidation, because derivatives of OMP are more stable. The oxidatively modified proteins content in the serum of female rabbits experimental group, measured as carbonyl oxidation levels, are shown in Figure 3. Liposomal preparation injection induced the decrease of carbonyl oxidation level (aldehyde derivatives) in the blood of the experimental group ($p < 0.05$) compare to that index in control group. Similarly, the ketogenic derivatives of oxidatively modified proteins level experimental animals was significantly lower ($p < 0.01$) than in control group.

CONCLUSIONS

The results of this study indicate that subcutaneous injection organic forms of trace elements in liposomal preparation 2 weeks before fertilization improves female rabbits' reproductive performance and is efficacious in enhancing implantation rates and also promotes the normalization of oxidation-antioxidant balance during pregnancy. The activity of antioxidant enzymes was significantly decreased by supplementation of liposomal preparation indicating improvement in the antioxidant activity and decrease oxidative stress to female rabbits during early state of gestation.

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