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PLASMA MACRO AND TRACE ELEMENT LEVELS OF MALE RATS VACCINATED WITH GNRH HORMONE

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ABSTRACT

GnRH hormone is important in both male and female reproduction. Chemical agents are preferred to prevent fertility in some animals. Trace elements play an important role in reproduction. In this study, it was aimed to investigate the changes in macro- and trace element levels in blood plasmas of 6th and 12th months of GnRH vaccination (Repro-Bloc ™) male rats. 50 male Wistar Albino rats were used in the study. Each rat in the experimental group was administered a single 200 µl intramuscular dose of GnRH. The levels of Cu, Fe, Mn, Zn, Se, K, P, Ca, and Mg elements were determined by analyzing the collected plasma samples using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

It was determined that zinc, manganese and selenium levels of the trace elements showed statistically significant changes in the first 6 months and Cu and Fe levels were statistically significant ($P<0.05$). In the twelfth month, it was observed that the levels of Fe, Mn, Se and Zn trace elements differ statistically. In the macro elements, K, P elements in the first 6 months period and Ca, Fe and Mg element levels in 16 months period gave statistically significant results ($P<0.05$). Conclusions: It was observed that levels of macro and micro elements were affected in male rats administered GnRH vaccine for immunocastration

KEYWORDS:
Trace element, Macro element, GnRH vaccine, Immunocasration, ICP-OES

INTRODUCTION

Hormones are vital chemical substances that affect many important events such as body weight, metabolism, appetite, growth, development and reproductive activities. The natural hormone, GnRH, is synthesized from the hypothalamus and stimulates synthesis and secretion of the gonadotropic hormones (FSH and LH) from adenohypophysis. Therefore, this hormone is important in both male and female reproduction [1].

Recently, there are unwanted increases in the number of domestic and wild animals. Therefore, the use of effective and safe contraceptive methods has become mandatory [2].

Many animal owners prefer chemical agents to chirurgical methods in terms of reliability, efficiency and cost to prevent unwanted mating. These methods prevent fertility from being recycled.

Hormonal applications involving progestogens, androgens, and the use of Gonadotropin Releasing Hormone (GnRH) analogues show the effect of discontinuing the direct hormonal-receptor relationship or indirectly controlling the reproduction through negative feedback [3].

It is stated that the vaccine developed against GnRH hormone showed its effect by reducing or preventing the secretion of gonadotropic hormones. Reduction or inhibition of secretion of gonadotrophic hormones causes atrophy of gonadal tissues, inhibition of gametogenesis, and therefore inhibition of reproductive behaviour. Vaccination of an individual after vaccination remained sterile for an average of 12 months, circulating immunoglobulin level decreased after the reproductive ability gained again was expressed. It has been claimed that immunocontraceptive vaccines, which allows return to the fertile period, having no systemic side effects, by providing success in one application and are relatively inexpensive compared to other methods [2].

Although high concentrations of heavy metals have negative effects on all living things, the effects of heavy metals and trace elements on reproductive capacity have not been fully explained [4]. Trace elements can cause beneficial and harmful effects on animal and human life due to their concentration. Trace elements, which are not possible to be synthesized by our bodies, must be taken from the outside from the basic food elements. Cell protection is important for healthy bone and skin structure. Trace elements also play an important role in blood pressure, heart rhythm, muscle function, maintaining fluid balance in the body, reproduction and many other functions. Scientific studies have shown that mineral loss and deficiency directly affect health. These elements are important because they act as antioxidants in the body, as they are cofactors of various enzymes and because they are involved in assimilation. In addition, metalloenzyme and metalloproteinase are involved as structural components and as stabilizers in

the membrane [5].

In this study, a GnRH vaccine (Repro-Bloc™) obtained in combination with cytosine phosphodiester guana oligodeoxynucleotide (CPG ODN) for the recombinant antigenic ovalbumin-GnRH fusion protein molecule in non-chirurgical control of reproduction in male rats was administered in the sixth year of the study and at 6th and 12th months, it is aimed to investigate the changes in the macro and trace elements.

MATERIALS AND METHODS

Animal material. Male rats, weighing about 3-4 months and 250-300 grams, were obtained from Van Yüzüncü Yıl University Experimental Animals Unit.50 male rats in the study were divided into 2 groups as 35 experimental groups and 15 control groups. For the study, permission was obtained from the Local Ethics Committee of Van Yüzüncü Yıl University in accordance with the decision numbered 03, dated 31.03.2011.

Application of the vaccine: at the beginning of each study of rats in the experimental group, from the GnRH Vaccine in the injectable form (Repro Bloc™), with a volume of 200 µl (Amplicon Vaccine, the single dose recommended by L.L.C.) was administered in a single dose intramuscularly. 15 rats in the control group were also injected into the muscle as sterile serum physiologic (ph: 7.2) as much as the volume of the vaccine.

Blood Collection. Intraperitoneal injection of ketamine (Ketalar, PFIZER) was applied before the blood was taken and general anaesthesia was applied. After anaesthesia, blood was taken from the heart of rats into the EDTA tubes. Blood samples were centrifuged for 20 minutes at 1600 rpm at 4 ° C in a refrigerated centrifuge to separate blood plasmids for analysis. Blood collection was carried out in 6th and 12 th months. The obtained plasma samples were stored at -20 ºC until the analysis of macro and trace elements was carried out.

Biochemical study. Papageorgiou et al.'s [6] method was used with some modifications. The plasma samples were taken into 1ml glass tubes and centrifuged in 2000-3000 rpm by adding 3 % HNO3 solution from 65 % HNO3 (Merck, Germany) and 1mlwas added. The remaining particles in the tubes were drained and then added 1ml 1 % Triton-X and the volume was completed to 10 ml with deionised pure water. The levels of Cu, Fe, Mn, Zn, Se, K, P, Ca, Mg elements were analyzed with ICP-OES (inductively-coupled plasma-optic emission spectroscopy) Thermoscientific ICAP 6000 series (0.005 ppm detectable limit) device.

Statistical analysis. descriptive statistics for continuous variables in our study were expressed as mean and standard deviation. Mann Whitney U test was used to compare the group mean of continuous variables. Statistical significance level in calculations was taken as 5 % and SPSS for calculations (IBM Corp. Released 2013). IBM SPSS statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) statistical package program was used.

RESULTS

Levels of Cu, Fe, Mn, Zn, Se, K, P, Ca and Mg were determined in plasma samples obtained from the blood taken at 6th and 12th. months for male rats and control groups applied GnRH vaccination.

In the sixth month, the levels of Cu and Fe trace elements in plasma samples showed statistically significant differences compared to the control group. P and K levels were determined to be statistically significant as macro elements (Table 1) (Figure 1-2).

In the twelfth month, it was observed that the levels of Fe, Mn, Se and Zn trace elements differ statistically. The levels of CA and Mg, among the macro elements, were determined to be statistically different (Table 2) (Figure 1-2).

TABLE 1
Macro and trace element levels at 6 months in male rats (µg/ml)

	Control	Experiment
Cu	1,100±0,670	0,697±0,129*
Fe	1,346±0,067	1,204±0,686*
K	141,338±86,039	108,410±30,694*
Mg	4,642±2,095	4,438±1,449
Mn	0,192±0,116	0,175±0,077
P	132,284±61,053	102,928±20,438*
Se	0,142±0,053	0,091±0,035
Zn	0,109±0,092	0,156±0,115

***p<0.05**

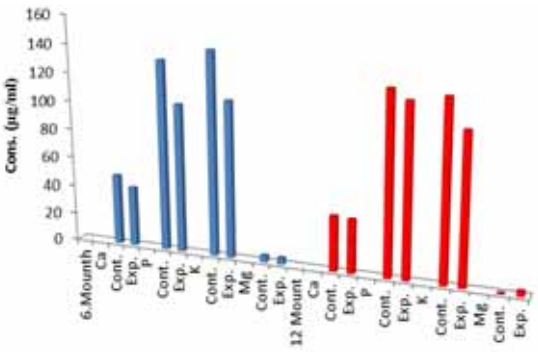


FIGURE 1
Change of macro elements at 6th and 12th months.

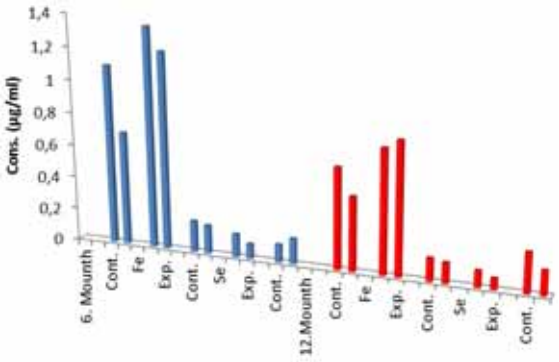


FIGURE 2
Change of trace elements during 6th and 12th months.

TABLE 2
Macro and trace element levels at 12 months in male rats (µg/ml)

	Control	Experiment
Cu	0,612±0,133	0,449±0,115
Fe	0,748±0,461	0,800±0,146*
K	122,454±17,891	102,480±36,235
Mg	0,158±0,336	3,434±1,926*
Mn	0,140±0,001	0,124±0,053*
P	124,924±18,206	118,000±32,271
Se	0,100±0,001	0,067±0,038*
Zn	0,242±0,367	0,148±0,063*

***p<0.05**

DISCUSSION

Trace elements are very important elements in the organism that are involved in many biochemical and metabolic reactions and are necessary for the immune system. The need for trace element in animals varies with such factors as age, production level, pregnancy and lactation period [7]. Macro and trace elements must be present in the organism for normal growth and continuity of life. The level of trace element in the metabolism is very important in the hormone system. There is an important relationship between hormones and trace elements. Both trace elements are involved in hormone metabolism, and disturbances in hormone function can alter the levels of trace elements in the blood and tissues [8].

In a study of plasma mineral (CA, P, mg, Zn, Cu, Co, Fe and Mn) levels investigated by applying Buserelin acetate to buffalos in pre-adolescence period it was found that only CU and Co levels were changed [9]. In a study in which intracytoplasmic sperm injection was performed with GnRH antagonist, serum and follicular fluid (Cd, Pb, Hg, As, Cu, Zn, Fe) levels were determined. It was determined that Pb's blood concentrations and Cu's follicular liquid concentrations may have significant effects on the reproductive cycle [10]. Maldonado et al. [11], in the study of reproductive and follicle development of cows, by injecting both trace element (Cu and Zn) and GnRH to Holstein cows, concluded that trace element injection may be a suitable way to increase the rate of pregnancy in cows in follicular and Corpus luteum development. Another study conducted in this area examined the changes in endocrine system by applying Zn, Cu, Mn and Co elements in different doses to the bulls in the prepubertal period. In the present study, it was found that the increase in testosterone concentration in each group and the LH ratio in pubertal bulls decreased compared to prepubertal bulls [12]. Zinc is one of the most important elements in this work involved in hormone metabolism [8]. Our work has resulted in such a way as to support this situation.

Copper is also the third major trace element found in the body after iron and zinc. Changes in the activity of some hormones are also known to affect copper metabolism [13]. A study of changes in trace element (Zn, Cu, Mn, Fe) levels of goats with follicular atresia in their ovaries was conducted. In this study, it is stated that trace elements provide basic data that can be used in the fertility improvement plan [14]. Phosphorus (P), after calcium, is the second largest element in the body. Phosphorus is a macro-nutrient that is responsible for the metabolism of carbohydrates, proteins and fats [15]. Testosterone can cause P and K to be retained. It can cause sodium and water retention depending on the pressure of testosterone and it can be expected to decrease by P and K level [16]. in our study, Cu, K and P levels decreased significantly in the 6 months after GnRH vaccination compared to the control group ($p<0.05$). This difference has been eliminated in the 12 months period, when the effect of the vaccine decreased. It was found that Plasma Fe level decreased in the 6 months period and increased in 12 months period.

This may well indicate that an important element such as Cu, which is particularly effective in the electron transport system, is suppressed under the influence of the GnRH vaccine.

At the end of the 12 month period, Zn, Se and Mn levels decreased significantly. This shows how effective Zn is on the metabolism of reproductive hormones in accordance with other literature. It is known that zinc is found in more than 2,000 proteins involved in gene expression by more than 300 enzymatic reactions [8].

In a study performed by Adedara et al. [17] through applying Mn and main elements to rats, pituitary-gonad suppression was tried to be done. In the study, it was found that there was a significant decrease in sperm quantity and quality in all rats. In a study to determine the link between concentrations of trace element (Zn, Cu, Mn, Fe, Co) and the release of testosterone in male rabbits, the protection of trace element levels was found to be important for both human and animal fertility and infertility problems [18]. In another study, it was concluded that trace elements (Cu, Co, Zn, Fe, Mn) should be taken in daily

nutrition in order to meet the need for trace elements for healthy growth and reproduction [19]. Manganese, glutamine synthetase, pyruvatedcarboxylase, as well as zinc as a cofactor are also found in the composition of enzymes such as superoxide dismutase. The superoxide dismutase containing Mn protects the cell against carcinogens caused by chemicals and radiation. Growth retardation, structural and chemical abnormalities in bones, infertility in females and disorders in lipid metabolism were observed in the lack of Mn in laboratory animals; [20]. In our study, it was observed that Mn levels in accordance with other literature showed significant changes at the end of 12 months.

Selenium, commonly found in nature, is one of the essential trace elements required for the normal development of human and animal organisms. In some studies, changes in hormone activity caused significant differences in selenium levels [21]. A study was conducted on the effects of selenium deficiency on testicular morphology and function in rats. In this study, it was determined that testicular morphology and function were influenced by selenium deficiency and that selenium element is necessary for testosterone biosynthesis, spermatozoa formation and normal development [22]. A study was conducted on how the application of pinealectomy and melatonin affects the elements in the blood and various tissues of rats. In this study, it was shown that pinealectomy significantly disrupts the metabolism of elements in the blood and various tissues of rats, and melatonin administration may have a regulatory effect on body element metabolism [23]. In our study, it was observed that the Se element value decreased during the 12 months period. This shows how effective hormone metabolism is on the Se element.

Magnesium is a macro element that participates in nerve conduction, muscle contracture and more than 300 enzymatic reactions [24]. Magnesium is the fourth in terms of quantity in the body, and the second most common cation after potassium intracellularly. Although it is found in all the cells, it has a higher concentration in the bones, muscles and soft tissues [24]. A study of the levels of trace elements in tissue in patients with myoma and uterine cancer shows that there was a significant increase in CA and Mg concentration in uterine cancer tissue compared to normal uterine tissue [25]. Chandra et al found that the addition of Mg increased the level of androgenic enzymes and testosterone [26]. In our study, Mg was significantly increased in 12 months.

Calcium is the most important element in the body and must be taken. It is also a cofactor of many enzymes. Testosterone increases the total amount of bone main substance (matrix), besides providing calcium accumulation. It is believed that the increase in the matrix is due to the anabolic effect of testosterone and the increase in Ca storage is due to the proliferation of bone matrix [16].

In the present study, it was observed that the level of Ca in the 12-month period was lower than the level of control and that it was influenced by GnRH hormone. In a study in which AI + GnRH (20μg) mixture was applied to cows for 3 months, the macro (Ca, P, Mg) and micro (Zn, Cu, Fe, Co, Mn) element levels were investigated and only Zn and Cu levels were changed [27]. The fact that the results of the studies we give in the light of this data are meaningful according to the results of the other studies may be due to the longer follow-up and the higher dose used.

CONCLUSIONS

As a result of the study, it was found that statistically significant changes were observed in trace elements for Cu, Fe levels at the 6th month after single dose GnRH and at Se, Zn Mn, Fe levels at 16th month. In addition, the macro elements K, P levels in the 6th month, and Ca, Mg levels in the 16th month produced statistically significant results. As a result, GnRH vaccine may be thought to have an effect on macro-element and trace element levels, and, depending on the dose and duration of use, interventions to the hormone system may cause long-term effects on metabolism.

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