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THE CHANGE OF BIOMETALS CONTENT (Cu, Mg, Zn) IN BLOOD AND SEMEN AND THEIR RELATIONSHIP TO THE FERTILITY OF MEN, WHO WERE CONTAMINATED BY ANTHROPOGENIC AIR POLLUTION

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This work is devoted to the research of the influence of the change of biometals content (Cu, Mg, Zn) in blood and seminal plasma and their relationship to the fertility of men, who were contaminated by anthropogenic air pollution. Such methods were used: cytochemical, biochemical and ecohygienic. The content of Zn, Mg, Cu in the blood and sperm of 190 men, 134 of them were metallurgical workers, was researched. We separated them according to the groups with such signs: fertility, contamination by air pollution and smoking.

The results of the research are the following: 1) air pollution influences on the content of chelatable metals in plasma of blood and semen and in the spermatozoons; 2) chronic influence of this factor decreases Zn and Mg content in blood and seminal plasma and in the spermatozoons, but this process is accompanied with increase of Cu content in fluids and cells; 3) the decrease of Zn and Mg content in fluids and cells leads to the result of an infertile status of the men who were contaminated by anthropogenic air pollution. In future we will study the methods of correction of this effect.

Key words: content of biometals in blood and seminal plasma, fertility of men, anthropogenic air pollution.

ЗМІНИ ВМІСТУ БІОМЕТАЛІВ (Cu, Mg, Zn) У ПЛАЗМІ КРОВІ І СПЕРМІ ТА ЇХ ВПЛИВ НА ФЕРТИЛЬНІСТЬ ЧОЛОВІКІВ, ЩО ПІДДАВАЛИСЯ ДІЇ АНТРОПОГЕННО ЗАБРУДНЕНОГО ПОВІТРЯ

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Еколого-гігієнічними та цитохімічними методами проводилися дослідження впливу змін показників металолігандного гомеостазу (вмісту Zn, Mg, Cu в плазмі крові та спермі і в сперматозоїдах) та фертильність чоловіків, що контактують з антропогенно забрудненням повітрям (пил важких металів, тютюновий дим). Обстежено 190 робітників металургійних підприємств, поділених на групи за ознаками: фертильність, контакт з важкими металами та паління.

У результаті дослідження було встановлено, що вплив антропогенно забрудненого повітря підвищує вміст Cu в плазмі крові і спермі, а також у сперматозоїдах, зокрем, за всіма показниками знижується вміст Zn та Mg, що у свою чергу викликає порушення чоловічої фертильності, яке проявляється зниженням фертильності в групі, що перебувала під дією антропогенно забрудненого повітря та до повного зникнення фертильності в групі курців, що контактували з пилом важких металів.

Подальші дослідження необхідно спрямувати на знаходження способів попередження виникнення цих явищ, а при їх виникненні – засобів їх корекції. Також бажано перед цим дослідити вплив антропогенних факторів на експериментальних тваринах.

Ключові слова: вміст біометалів в плазмі крові та спермі, фертильність чоловіків, антропогенно забруднене повітря.

ИЗМЕНЕНИЯ СОДЕРЖАНИЯ БИОМЕТАЛЛОВ (Cu, Mg, Zn) В ПЛАЗМЕ КРОВИ И СПЕРМЕ И ИХ ВЛИЯНИЕ НА ФЕРТИЛЬНОСТЬ МУЖЧИН, ПОДВЕРГАВШИХСЯ ВОЗДЕЙСТВИЮ АНТРОПОГЕННО ЗАГРЯЗНЁННОГО ВОЗДУХА

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Эколого-гигиеническими и цитохимическими методами, проводилось исследование влияния изменений показателей металлолигандного гомеостаза (содержание Zn, Mg, Cu в плазме крови и сперме, а также сперматозоидах) на фертильность мужчин, контактировавших с антропогенно

загрязнённым воздухом (пыль тяжёлых металлов, табачный дым). Обследованы 190 рабочих металлургических предприятий, разделённых на группы по признакам: фертильность, контакт с тяжёлыми металлами и курение.

В результате исследования установлено, что воздействие антропогенно загрязнённого воздуха повышает содержание Cu в плазме крови, сперме, а также в сперматозоидах. Кроме того, происходит снижение содержания Mg и Zn по всем показателям, что в свою очередь вызывает нарушение мужской фертильности мужчин, сочетавших курение с воздействием загрязнённого воздуха.

Дальнейшие исследования необходимо направить на нахождение способов предупреждения возникновения этих негативных проявлений, а при возникновении – методов их коррекции, желательно предварительно отработанных на экспериментальных животных.

Ключевые слова: содержание биометаллов в плазме крови и спермы, фертильность мужчин, антропогенная загрязнённость воздуха.

INTRODUCTION

On the biological trace elements Zn, Mg and Cu are important in reproduction. Zinc has been extensively studied. Its deficiency leads to gonadal dysfunction [1-3] decreases testicular weight and causes shrinkage of seminiferous tubules [4-7]. Some of the zinc deficiency states such sickle cell anemia, chronic alcogolism, idiopathic male sterility, or toxic effects of di-(2-ethyl hexyl)-phthalate (DEHP) or other phthalic acid esters (PAEs), cause atrophy of the testis and the atropty is attributed to low availability or increased urinary excretion of zinc [1-7]. Zinc deficiency is also linked to malignant growth in the testis. Zinc content is high in the adult testis compared to immature animals or those in which the efferent duct is ligated. Zinc concentration of the men increases at puberty and reaches a maximum at the age of 34-40 years of age when functional activity of the organ is at its peak. Similarly the rats with a maldescended testicle, the ectopic testis has a decreased zinc content, whereas that of the other testicle is normal. Zinc deficiency impairs the action of the Mullerian inhibitory factor which is essential for testicular differentiation [1-4].

Angiotensin converting enzyme (ACE) is closely associated with testicular development and sperm development [1-7]. ACE is primarily localized in the germinal cells and has very little activity in Leydig and Sertoli cells. The precise role of ACE is not clear yet but reduced ACE activity in the testis of zinc-deficient rats has been analyzed [1, 3, 4]. It is believed that zinc deficiency first impairs ACE activity, and that in turn leads to depletion of testosterone and finally impairs spermatogenesis. Although the probable site of action is considered to be primary spermatocytes, it has also sperm heads [1, 8, 9].

Zinc appears to be an indispensable element in reproduction for another reason too. The gonads are the most rapidly growing tissues in body and vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes [1-4].

The three zinc cations, cuprum and magnesium stimulated or inhibit progressive motility depending on the concentration of each. At high concentration, these elements, individual or jointly, impair fertility in the patients with normal sperm density. Although a positive correlation between seminal fluid, zinc and sperm motility and sperm density of asthenozoospermic men is studied excessively in asthenozoospermia. Some of the important enzymes of spermatozoa are zinc metalloenzymes and can thus become dysfunctional when zinc is deficient [1-7].

Spermatozooon zinc is suggested to protect an inherent capacity of decondensation, thereby assist to extend the functional life-span of the ejaculated sperm. The spermatozoon head accumulates a fourfold higher zinc concentration than seminal plasma, and the high-affinity zinc bilding sites are present within the nuclear matrix. Zinc is related to the structural integrity of DNA, and prevents destruction of DNA by inhibit degrading enzymes [1-6].

Further, an insufficient zinc level in the nucleus may destabilize the quaternary structure of chromatin, reduce the DNA content of spermatozoa and thereby reduce their fertilizing capability [1-3].

The concentration of zinc (Zn) in human seminal plasma is higher than in other tissues. Zinc is the metalloprotein cofactor for DNA-binding proteins with Zn fingers. It is part of cooper (Cu)/zinc superoxide dismutase and several proteins are involved in the repair of damaged DNA (which are mutated in half of human tumors) and in the transcription processes of DNA [1, 3, 8]. Zinc has an important role in testes development, sperm physiologic functions and decreasing of its levels causing hypogonadism, decrease in testes volume, inadequate development of secondary sexual characteristics and atropy of seminiferous tubules (and hence spermatogenesis failure). Recent studies hypothesized that insufficient intake of Zn can impair and make the sperm cell hidhly susceptible to oxidative damage [1-7].

Infertile and smoker men are very susceptible to oxidative damage induced by free radicals. High levels of free radicals may overwhelm the antioxidant strategies (especially the effective concentrations of seminal Zn), which is associated with low quality of sperm. In the present study, we hypothesized that major changes in the level of seminal trace elements, especially Zn levels, are related to low quality of sperm and poor fertilizing capacity. Therefore this study is focused primarily on Zn levels in the seminal plasma of fertile and infertile subjects (smokers and nonsmokers). The connection of Zn and a certain degree of Mg in the seminal plasma of all groups was evaluated [1-4].

Zinc is also localized in the Golgi complex or secretory vesicles of interstitiotrophs (IT), folliculotrophs (FT) and lactotrophs (LT) of the pituitary gland. Thus it seems that the element plays an important role in the production and secretion of LH, FSH and prolactin, and these in turn regulate testosterone production. It is now well-known that zinc deficiency depresses steroidogenesis [1, 3, 4]. Besides its effect on androgen metabolism, it interacts with steroid receptors and androgen binding protein. The earliest citochemical changes occur in Leydig and Sertoli cells in mice after two weeks of zinc deficiency. Cholesterol and neutral lipids are precursors of sex steroids. Under the zinc deficiency they are uptaken by the germinal and non-germinal cells of the testis that does not seem to be affected, but probably the cells are incapable of converting them into sex steroids. This is thought to be the reason that there is a low level of serum testosterone, in spite of high serum LH and FSH levels, after LHRH injection to zinc-deficient rats. Sertoli cell products hormone (SCH) forms the metabolism of testosterone, triggers the formation of new cell lines in the testis, its insufficiency, because of defective metabolism of cholesterol, appears to be responsible for the arrest of spermatogenesis [1-7].

The main aim of this work is the study of the influence of air pollution containing hydrogen sulfide of toxic metals to the content of trace elements (Zn, Mg, Cu) in blood and seminal plasma and their relationship to fertility of the men who were contaminated by anthropogenic air pollution.

MATERIALS AND METHODS

Biochemical methods were used to estimate Zn, Mg, Cu content in blood and sperm for mass investigations of industrial workers. The methods are relatively complicated and require great quantities of blood sperm samples. The citochemical methods used here are simple and need relatively small amounts, only a few drops are required for each analysis.

The cells of 190 subjects were investigated in total, with 56 people as controls, unexposed to the air pollution. Exposure of 134 workers to hydrogen sulfide and toxic metals (in metallurgical facilities) was studied. Significant exceeding of hydrogen sulfide concentration of air to 1,4 maximum allowable concentration (MAC) was noted. Average annual indicator of hydrogen sulfide in working zone air formed 0,8 MAC. Toxic metals concentration in working zone air of metallurgic industry fluctuated within the range from 0,7 to 1,0 MAC.

All participants were within the age range of 24-35 years. Almost none of them had acute infections at the time of testing. The period of exposure was one year. The exposures were similar in each investigated department.

Procedures. All the subjects were interviewed by an experienced investigator using a questionnaire to obtain information on occupation exposure at work to metals, general health, living habits, including cigarette smoking and alcohol drinking, and medical history. In addition, a medical examination was conducted by an andrologist.

The subjects were asked to collect semen at home in the morning by masturbation into a sterile wide-mouth plastic container after at least three days abstinence. The samples were brought to the hospital within an hour of collection. Time of ejaculation, abstinence period, and spillage (if any) were recorded by the subjects; 10 ml of venous blood was also drawn from the subjects into a heparinized plastic tube on the day when they handed in the semen samples. Semen screening was performed as soon as possible after the specimen was delivered at the Seminology laboratory of the reproductive clinic. The semen screening included semen volume, sperm density, motility, morphology, and viability. These were performed according to the World Health Organization guidelines for examination of human semen. The blood and semen samples were stored in metal free containers and kept at -40ºC and 4ºC respectively before analysis.

Semen samples were obtained by masturbation into a sterile container after sexual abstinence for 2 to 3 days. Before semen analysis, a questionnaire was distributed to obtain information on smoking habits; alcohol use, use or abuse of other substances and drugs and a history of orchitis, testicular trauma, sexually transmitted disease, varicocele, surgery for inguinal hemia and cryptorebism. Consent was obtained from the subjects. Fertile and infertile patients who smoked cigarettes regularly or who were nonsmokers (previously never smoked) were included in the study [1, 5-9].

Semen parameters analysis. After collection, semen specimens were allowed to liquefy at room temperature for 30 minutes and used for analysis. On microscopic examination, sperm and sperm with normal morphology were objectively evaluated according to standards set by the World Health Organization. Sperm morphology was evaluated according to the criteria by Kruger [1, 5-9].

Measurement of Cu, Mg, Zn in semen and blood plasma. Semen samples were centrifuged at 600 g for 10 minutes. After centrifugation, supernatants were diluted 10-fold by deionized water. Levels of Cu, Mg, Zn were measured by atomic absorption spectrophotometre [10].

Measurement of Cu, Mg, Zn in spermatozoons

Seminal smears were fixed for 5 min in formalin vapors before dithizone staining. 0,2% water – ammonia solution of this reagent was used. The dye was prepared in the following way. Combination of 45 ml of distilled water, 600 mg of dithizone, and 0,9 ml of 25% solution of ammonium hydroxide were placed into a flask and mixed in water bath at 70ºC during 10 min. Then the content was filtered and 1% water ammonia solution of dithizone was obtained because the fourth part of reagent remained insoluble. Working solution of dithizone was prepared by fivefold dilution of basic. (1%) solution with 0,2% solution of natrium diethyldithiocarbamat. Reagent of Mg was lumomagneson, of Cu lumocuppheron metals content in spermatozoids was evaluated with semi – quantitative and quantitative methods.

The first one was carried out using the following criteria: 1 = weak, 2 = moderate, 3 = pronounced reaction intensity. The score for 100 cells was counted, using colored scales [6]. The quantitative method was based on granule number score in the cells. The number of spermatozoids was expressed as its number per 1 mμl of the semen. The results are presented as $\overline{X}$ +SD; testing for significance is performed using Students' t-test.

RESULTS AND DISCUSSIONS

Average values of sperm parameters in fertile and infertile groups can be observed in Table 1. No significant differences between the groups in age and semen volumewere observed. Sperm quantity, motility, and normal morphology in fertile group (smokers or nonsmokers) were significantly higher than those in infertile group. A trend toward a higher quality of sperm was seen for nonsmokers compared with smokers (Table 1).

Average concentrations of Zn, Mg, Cu in the seminal plasma of all samples can be seen in Table 2. The concentration of elements in seminal plasma was in the order Cu, Zn, Mg. No significant differences were observed in average concentrations of Zn, Mg and Cu between groups. However, a trend (P = 0,9) was observed for a lower average Mg levels in seminal plasma of infertile smokers compared with infertile nonsmokers. Moreover, a trend (P = 0,7) toward higher Mg levels was observed in seminal plasma of fertile nonsmokers compared with infertile nonsmokers (Table 2).

Fertile groups (smokers or nonsmokers) demonstrated significantly higher Zn levels in their seminal plasma than any infertile groups (P< 0,001). A trend was observed for a lower average Zn levels in seminal of smokers compared with nonsmokers was observed (Table 2). Fertile nonsmokers had significantly higher levels of Zn in their seminal than infertile nonsmokers (P< 0,001), moreover fertile smokers had significantly high levels of Zn in their seminal plasma compared with infertile smokers (P< 0,001). A trend (P = 0,7) for a lower average Zn levels in seminal plasma of fertile smokers compared with fertile nonsmokers was observed. Moreover, there was a trend (P = 0,6) toward higher average Zn levels. Average content of metals in spermatozoons in 2 studied groups can be observed in Table 3.

Table 1 – Age and semen parameters in 2 studied groups.

Variable	Fertile group		Infertile group	
	control (n = 56 nonsmoker, unexposed by the air pollution)	nonsmoker, exposed by the air pollution (n = 38)	nonsmoker exposed by the air pollution (n = 46)	smoker, exposed by the air pollution (n = 52)
Age (y)	31,25 ± 4,02	29,43 ± 3,6	29,63 ± 3,38	31,01 ± 4,53
Volume (ml)	4,53 ± 1,15	4,21 ± 1,35*	4,11 ± 1,61	3,2 ± 0,82
Sperm content ($\times 10^6$)	87,58 ± 4,95	77,59 ± 16,35*	38,29 ± 15,9$^{\$}$	32,02 ± 18,97†
Total sperm ($\times 10^6$)	384,25 ± 15,32	345,53 ± 137,6*	157,3 ± 13,58$^{\$}$	95,38 ± 6,48†
Motility (%)	65,29 ± 8,69	63,94 ± 7,85*	41,93 ± 5,14§	41,87± 5,25$^{\#}$
Normal morphology (%)	16,25 ± 4,01	12,31 ± 3,92*	5,7 ± 1,49$^{\$}$	5,3 ± 0,5***

Note: Values are presented as average ± SD;

* P < 0,05 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of fertile smokers are compared with fertile nonsmokers;

*** P < 0,001 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of fertile smokers are compared with fertile nonsmokers;

$^{\$}$ P < 0,001 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile nonsmokers.

† P < 0,01 by using 1-way analysis of variance followed by post hoc Newnan-Keuls test when values of infertile smokers are compared with fertile smokers.

§P < 0,01 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile smokers.

$^{\#}$P < 0,01 by using 1-way analysis of variance followed by post hoc Newman-Keuls test when values of infertile nonsmokers are compared with fertile smokers.

Table 2 shows relevant data of 56 referenced subjects and 136 workers who were exposed by the air pollution. The referenced group included 38 fertile nonsmoker exposed by the air pollution (I g), 46 infertile nonsmoker exposed by the air pollution (II g) and 52 infertile, smokers exposed by the air pollution (III g). The incidence of the control group may explain higher content of Zn, Mg and lower content of Cu in blood and seminal plasma.

The incidence of the group (III g) may explain lower content of Zn and Mg and higher content of Cu in blood and seminal plasma.

Table 2 – The content of metals (Zn, Mg, Cu) in blood and seminal plasma in 2 studied groups

Variable	Fertile group		Infertile group	
	control (n = 56 nonsmoker, unexposed by the air pollution	nonsmoker exposed by the air pollution (n = 38)	nonsmoker exposed by the air pollution (n = 46)	smoker, exposed by the air pollution (n = 52)
Zn (in blood plasma, μmol/l)	12,25 ± 2,68	10,21 ± 0,84***	7,78 ± 0,99**	6,57 ± 1,63***
Zn (in seminal plasma, μmol/l)	135,85 ± 2,54	110,25 ± 12,25***	101,95 ± 2,05***	94,75 ± 2,5***
Mg (in blood plasma, mmol/l)	1,21± 0,07	1,05 ± 0,14***	0,75 ± 0,08**	0,3 ± 0,06***
Mg (in seminal plasma, mmol/l)	15,21 ± 2,67	12,75 ± 1,54	10,12 ± 2,65**	8,25 ± 1,33
Cu (in blood plasma, μmol/l)	15,45 ± 2,86	17,21 ± 2,14***	18,75 ± 1,86***	22,12± 3,95***
Cu (in seminal plasma, μmol/l)	175± 9,58	192,95 ± 1,85***	240,52 ± 2,75***	270,75 ± 14,85***

Note: ** P < 0,01;*** P < 0,001.

Table 3 – The content of metals (Zn, Mg, Cu) in spermatozoons in 2 studied groups

Intensity of cytochemical reaction, sing	Fertile group		Infertile group	
	control (n = 56) nonsmoker unexposed by the air pollution	nonsmoker exposed by the air pollution (n = 38)	nonsmoker exposed by the air pollution (n = 46)	smoker, exposed by the air pollution
Zn (dithizone)	1,5± 0,16 **	1,2 ± 0,14**	1,09 ± 0,09**	0,85 ± 0,08 ***
Mg (lumomagnesone)	1,01 ± 0,07 ***	0,92 ± 0,02***	0,74 ± 0,04***	0,32 ± 0,05**
Cu (lumocuppherone)	0,50 ± 0,05***	0,72 ± 0,06**	0,85 ± 0,02***	1,01 ± 0,04***

Note: *P < 0,05; **P < 0,01;*** P < 0,001

The content of Zn and Mg in spermatozoons decreased but the content of copper increased. This fact proves that Zn and Mg are synergists but Cu is their antagonist. The influence of contamination of atmospheric air had a negative effect on the characteristics of spermatozoons.

Table 4 shows the correlations between each of the measured reproduction parameters with respect to S_{Zn}, S_{Mg}, S_{Cu}, smoking age and exposure by the air pollution S_{Zn} and S_{Mg} the increase of quantity of total sperm, motility of sperm and the decrease of percentage of pathologic sperm. $S_{Cu,}$ smoking and exposure by the air pollution decreased these parameters.

Table 4 – The Spearmen rank correlation coefficient and the level of significance (r, p) for relationships between the parameters of semen quality with respect to zinc (S_{Zn}), magnium (S_{Mg}), cuppers (S_{Cu}), smoking habits, age and exposure by the air pollution.

Parameter	S_{Zn}	S_{Mg}	S_{Cu}	Smoking	Age	Exposure by the air pollution
Semen volume	0,145	0,151	-0,135	-0,004	0,035	-0,052
Sperm volume	0,291	0,318	-0,164	-0,009	-0,044	-0,210
Motilite sperm, %	0,152	0,148	-0,191	-0,028	0,076	0,001
Normal morphology, %	0,139	0,142	-0,079	-0,141	0,025	-0,018

1. Air pollution influences on the content of chelatable metals (Zn, Mg, Cu) in plasma of blood and semen in the spermatozoids.

2. Chronic influence of this factor decreases Zn and Mg content in the blood and seminal plasma and in spermatozoons, but this progress is accompanied with the increasing of spermatozoons.

3. The decreasing of Zn and Mg content in blood and seminal plasma and spermatozoons leads to an infertile status of the men, who were contaminated by anthropogenic air pollution. In future we will study the method of correction of this effect.

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MODULATED ELECTRIC CURRENT INFLUENCE ON ORGANISM FUNCTIONAL CONDITION AT TRAINED AND UNTRAINED YOUNG MEN

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The short characteristic of organism functional condition changes of trained and untrained young men during physical activity and processes of restoration is given in the paper following the analysis of repeated modulated electric current influence on the specified processes. The aim of the study was to compare the characteristics of a modulated electric current influence on cardiovascular system functional condition in trained and untrained persons (17-24 years old) during the physical activity and restoration.

The study included 56 healthy young men (23 trained and 33 untrained), who were divided into the main group (11 trained and 16 untrained persons) and control group (12 trained and 17 untrained persons). The study included two stages: the first stage – the baseline functional condition was estimated in both groups; the second stage – the functional condition state reaction to repeated modulated electric current during the