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The effect of cadmium and zinc content in sperm on spermogenesis parameters in male infertility

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Abstract

Relevance: Male infertility is a multifactorial disorder that occurs in about 50% of infertile couples. One of the reasons of infertility may be the increased exposure to toxic elements.

Purpose: to study the interaction of cadmium and zinc content in male sperm according to spermogram results.

Keywords: male infertility; zinc; cadmium; spermogram

Introduction

Infertility is now considered to be one of the major health problems in family planning system, which affects almost 10-15% couples worldwide [1]. Male infertility is a multifactorial disorder that occurs in about 50% of infertility cases [2]. In addition to anatomical-physiological, hormonal, infectious-immunological and molecular-genetic defects, gametogenesis and male fertility are also influenced by the lifestyle and environmental factors [3].

Zinc content is critical in proliferating tissues as well as in mobile cells. Zn plays role as a regulator of gametogenesis and steroid hormonopoiesis, as well as a mediator of protein and transport systems functioning. It is not only a cofactor of many enzymes which are involved in antioxidant protection and electron transfer, but it is also important for the production, storage, secretion and functioning of RNA polymerase, alcohol dehydrogenase, carbonic hydrase, alkaline phosphatase. Directed transport and control of its use is carried out with the participation of low-molecular transport proteins of metallothioneins, which are also actively involved in cadmium binding and excretion [4]. Numerous researches have shown a positive effect of zinc on sperm quality and treatment of male infertility [3]. It is also the main factor for DNA replication and packaging, DNA transcription, protein synthesis, cell proliferation, differentiation and apoptosis, which are the main stages of sperm development [5]. Zinc also plays a regulatory role in sperm formation and acrosis reaction [6].

Numerous researches report about high zinc concentration in the seminal plasma of healthy people, ranging from 50 to 500 mg/L [7, 8, 9]. Reduced zinc level in seminal plasma of patients are associated with abnormal sperm parameters. For example, Kothari and Chaudhari reported about decrease of seed zinc level in plasma in asthenoteratozoospermic, oligoastenoteratozoospermic and azoospermic samples compared to normozoospermic [6]. A large number of studies have demonstrated a disorder of spermatogenesis and sperm abnormalities in patients with zinc deficiency and nutritional disorders [4]. In contrast, several studies showed no significant differences in mean of zinc level in seminal plasma between fertile and infertile subjects [10]. The World Health Organization (WHO) informed that one third of the world's male population suffers from zinc deficiency [8].

Cadmium is highly toxic heavy metal, which is widely used in the industries connected with production of galvanic electrical engineering, polymer materials, batteries and enamel coatings, and along with, it is a global ecotoxicant [11]. Consequently, there is a high level of heavy metal contamination in many places in the world with poor environmental control systems, which eventually shows up in food chain and has a detrimental effect. A special groups of attention are employees of industrial enterprises with unfair working conditions, who are in direct contact with reagents and are also jeopardized by excessive toxins exposure on their health because of professional risk. Cadmium is similar to zinc by chemical and physical parameters (both are located in the same group of Mendeleev D. I. periodic system) and they are competitively resorbed through the digestive and respiratory tract in one's body [12]. The main source of cadmium input is manufacture connected with production, processing or disposal of nickel-cadmium batteries in case of non-compliance with labor protection conditions, as well as smoking. The level of cadmium in smokers exceeds the average population level of this microelement by 4-5 times [13]. In addition, contaminated drinking water and foodstuffs contribute an important role to cadmium exposure [14]. The latter factors are not relevant for the south of Ukraine, where the content of cadmium in food retail chain and drinking water is strictly normalized, strictly controlled and does not exceed the norm.

High level of cadmium intake has a significant adverse effect on cell growth rate [15. 16], but its toxic effects vary by tissue type and are observed primarily in the liver, kidneys and gonads. Researches of cadmium toxicity have shown that it is a carcinogen for humans and one of the most famous reproductive toxicants [17, 18].

Although various researches have shown lower zinc values in sperm of infertile men compared to subjects with normozoospermia [19], still the pathogenetic mechanisms of zinc deficiency in spermatogenesis disorder in male infertility have not been fully studied. Current researches have examined the interaction between zinc concentration and the quality of sperm parameters, but controversial results have been obtained. In addition, there is a limited information about the potential role of ecologically relevant cadmium impact in general population regarding reproductive men opportunities.

Purpose of research is to study the connection between the effects of absolute (nutritive deficiency) and relative (competitive cadmium impact) zinc deficiency in seminal fluid and the quality of sperm (concentration, morphology, viability and mobility) of men in general population, as well as the results of targeted replacement, detoxification and antioxidant therapy in the treatment of male infertility.

Materials and methods

108 clinical cases of male infertility have been studied with complex diagnosis at the University Clinic (UC) of Odessa National Medical University for the period from 2016 to 2019. The target selection was formed from a general variation series of patients diagnosed with primary or secondary infertility with preserved erectile function after excluding anamnestic and catamnestic data in favor of compromised state of the reproductive system (injuries, neoplasms, operative, chemotherapeutic or radiation treatment) and aggravated comorbid background (liver and kidney diseases, diabetes mellitus, obesity II and III art., medication).

The key points that determined the course of the research were: increase of cadmium content in the seminal fluid (> 1 μg/l), low zinc concentration (< 50 μg/l) and sperm quality indicators. The selection extent was sufficient enough to obtain 80% results with one-way variance analysis at a 5% significance value. All participants were fully informed about the purpose of the research and signed the agreement form. The research was approved by the bioethics committee of Odessa National Medical University.

The final selection consisted of 108 men with infertility. All patients were of working and childbearing age. The average age of patients is 33 years (maximum 58 (1 person), minimum 24). The age distribution is shown in Figure 1.

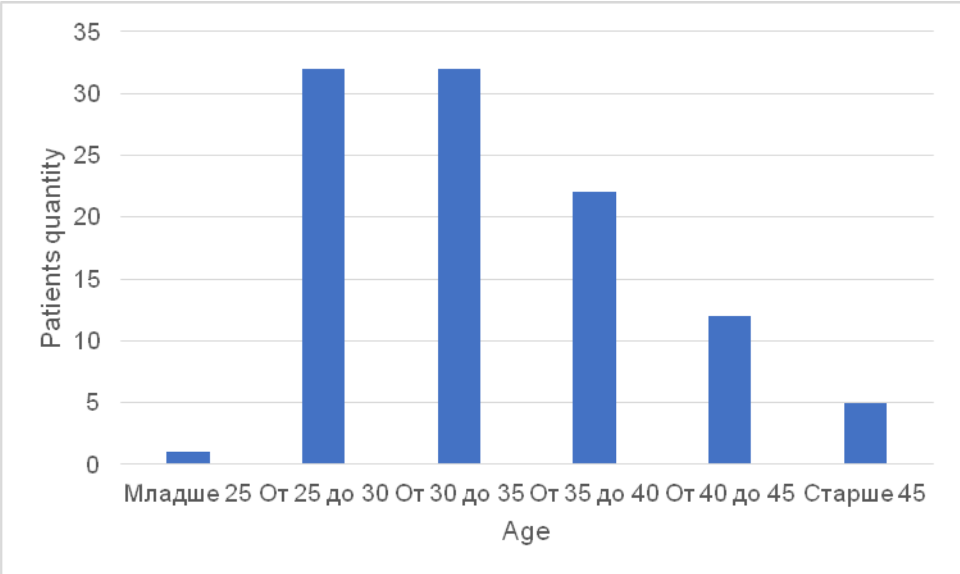

Fig.1. Age distribution of the patients

The total selection was standardized by anthropometric and clinical parameters. In all cases, high concentration of cadmium was observed in patients who are professionally employed in industries using this microelement; work experience was from 7 to 22 years, all patients underwent an annual medical examination and were further examined for specific somatic pathology.

There were 68 (62.96%) of surveyed people who smoked during the last 5 years and/or smoking currently. 83 people (76.85%) were drinking alcohol moderately (mainly beer). 82 people determined their diet as balanced, 26 people have never consumed fast food. All surveyed lived for at least 5 recent years in the conditions of a large industrial city.

Determination of metals in seminal fluid

Cd content was determined by atomic absorption method (along with electrothermal atomization) (ETAAS) and Zn atomic emission analysis (AES). The sample preparation was carried out by decomposing sperm samples in a microwave oven in concentrated nitric acid area in sealed Teflon vessels. State Standard Samples (SSS) were used to construct calibration graphs. The detection limits were: for Cd 0.1 μg/L, for Zn 0.1 mg/L.

Sperm quality analysis

Participants were informed of the need to refrain from ejaculation for at least three days before collecting semen. All samples were analyzed for sperm concentration and motility according to World Health Organization guidelines (WHO 2010). Percent of motility was estimated by calculating the percentage of motile sperm compared to immobile cells for 10 microscopic fields. To assess morphology, air-dried medications were stained according to Papanicolaou and classified as normal or abnormal according to WHO criteria (WHO 2010). The results were expressed as a percentage according to normal sperm. All procedures were performed by the same laboratory assistant. The average inter-individual variation coefficients were 13%, 11% and 14% for sperm concentration, motility and viability, respectively.

Luteinizing hormone (LH), prolactin PRL, follicle stimulating hormone (FSH), total and free testosterone, sex hormones binding globulin (SHBG) were additionally measured in blood.

The therapeutic tactic was: to isolate from harmful manufactures in cases with exceeded content of cadmium in the seminal fluid and/or blood, to normalize balance of work and rest, to change the diet with predominance of features of the Mediterranean diet (zinc-rich seafood, olive oil, fresh green vegetables), to abandon bad habits, and also pharmacological correction (equivalent to the microelements content): zinc medication (40 mg/day), selenium (200 μg/day), retinol (1500 μg/day), tocopherol (180 mg/day) during 3 months. The use of complex-forming substances was not carried out due to the cadmium content in the blood plasma below maximum permissible levels.

Statistical analysis

A descriptive analysis was performed in order to determine the average distribution of anthropometric parameters, sperm quality parameters and metal concentration in seminal fluid. The average concentration of each metal was calculated based on WHO reference values. The dynamics of metal concentration in the two categories of each sperm parameters were compared with use of χ^2 Pearson. The connection between categories for each metal and sperm concentration, mobility and viability was assessed by binary logistic regression, when subjects were dichotomized in categories lower or higher than WHO reference levels (concentration $> 15 \times 106$/ml, mobility $\geq 50\%$, and viability $\geq 50\%$, normal morphology $\geq 4\%$). Data analysis was performed using the XLSTAT for Microsoft Office Excel 2019.

Results

Before treatment

Table 1 shows the characteristics of participants stratified by sperm quality according to WHO reference values.

Table 1 shows statistically processed data on the chemical elements content in the sperm of infertile men.

Table 1

Statistically processed data on chemical element content in sperm of infertile men, *N* = 108

	Content, mg/L (µg/ml)	
	Zn	Cd
Contingent norm *	90-150	< 0,010
Average	116,6	0,0062
Minimum	39,4	0,0005
25% quartile	84,1	0,0032
Median	101,0	0,0046
75% quartile	136,4	0,0078
Maximum	366,4	0,0385

Note: the average data of own researches, scientific publications and reference literature

Cadmium and zinc content in sperm of infertile men in Odessa region differs slightly from data obtained earlier [20, 21, 22].

Table 2

Statistically processed data from spermograms of infertile men with escretor-toxic infertility, *N* = 108

	Liquefaction time, min	Viscosity, mm	pH	Number of sperm million/ml	Number of sperm in whole ejaculate, million	Quantity of mobile (cat. a + b + c),%	Quickly progressively mobile (cat. a)%	Slowly progressively mobile (cat. b)%	Rotary or oscillatory mobile (cat. c)%	Immobile (cat d)%	Viable, %	Leukocytes	Morphologically normal spermatozoa,%
Contingent norm	-	2	7 - 8	>20	> 39	> 50	-	-	-	-	> 50	< 2	>4
Average	40,7	0,45	7,95	116,1	355,8	57,7	26,97	21	11,1	41	58,3	7,3	27,6
Minimum	2,3	0,1	7,3	1	5	18	0	4	1	7	20	1	4
25% quartile	25,75	0,2	8	41,75	124	47,5	13	13	6	30	46,75	4	17
50% quartile (median)	30	0,3	8	84	291	59	24,5	20	10	40	59,5	5	24
75% quartile	42	0,4	8	159,5	477,5	70	37,25	28	15	50	68,25	8	35,3
Maximum	401	4	8,5	1161	2322	93	68	43	46	82	98	35	68

Data analysis of Table 2 shows that the sperm concentration in 1 ml varies widely from 1x106 to 2322 x106. At the same time, no significant correlation interaction between concentrations of chemical elements and the main parameters of sperm quality were revealed.

For detailed analysis of cadmium effect on sperm parameters, patients were ranked by cadmium content per 3 groups (< 5 ng/ml, > 5 ng/ml, from group > 5 ng/ml, patients with cadmium content more than > 10 ng/ml were separately isolated) and sperm parameters were statistically processed within-groups.

Table 3

Statistically processed data from spermograms of infertile men depending on cadmium concentration

	Av. (min.-max.)	Av. (min.-max.)	Av. (min.-max.)
	Cd<0,005 mg/l (mkg/ml) 56 people	*Cd>0,005 mg/l (mkg/ml) 52 people*	*Cd>0,010 mg/l (mkg/ml) 11 people*
Age	33,0 (25,0 - 58,0)	33,4 (24,0 - 51,0)	34,0 (24,0 - 58,0)
Zn, mkg/ml	120,7 (39,4 - 288,7)	99,3 (51,4 - 366,4)	84,3 (51,4 - 123,4)
Cd, ng/ml	3,2 (0,5 - 4,9)	8,4 (5,4 – 38,5)	16,9 (10,6 – 38,5)
Liquefaction time, min	34,1 (13,0 - 401,0)	33,8 (2,3 - 63,0)	39,1 (2,3 - 63,0)
Viscosity, mm	0,4 (0,2 - 1,3)	0,4 (0,1 - 4,0)	0,8 (0,2 - 4,0)
PH	8,0 (7,3 - 8,2)	8,0 (7,4 - 8,5)	7,9 (7,4 - 8,3)
Number of sperm million/ml	160,5 (16,0 - 1161,0)	131,7 (5,0 - 230,0)	91,4 (1,0 - 230,0)
Viable, %	60,9 (40,0 - 98,0)	52,3 (20,0 - 61,0)	47,1 (20,0 - 48,0)
Leukocyte	4,5 (2,0 - 14,0)	7,2 (5,0 - 25,0)	11,1 (9,0 - 35,0)
Morphologically normal sperm,%	27,5 (4,0 - 67,0)	24,7 (4,0 - 48,0)	15,3 (11,0 - 32,0)
LH	4,1 (1,3 - 7,7)	3,8 (1,2 - 7,7)	3,8 (1,2 - 6,7)
PRL	35,2 (4,7 - 291,0)	31,6 (3,5 - 235,0)	38,6 (5,4 - 164,0)
FSH	3,9 (1,7 – 9,5)	4,5 (1,8 - 10,5)	5,2 (2,2 - 12,0)
General testosterone	15,5 (2,5 - 34,8)	17,9 (3,0 - 36,8)	22,0 (3,0 - 33,1)
Free testosterone	14,7 (0,1 - 49,9)	15,4 (0,1 - 62,3)	26,5 (2,1 - 62,3)
SHBG	32,0 (7,4 - 63,2)	30,9 (6,0 - 84,3)	33,6 (6,0 - 84,3)

The range of main indicators are crossed in many areas, but if to compare the distribution of main indicators within each group, the result becomes more clear and demonstrative.

Table 4

Distribution of spermograms of infertile men with escretor-toxic infertility according to cadmium concentration before the treatment

Spermogram parameteres	% of examined men		P χ^2 Pearson
	Cd<0,010 mkg/ml, %	*Cd>0,010 mkg/ml, %*	
Concentration			
> $15x10^6$ / ml	93,47 %	58,33%	< 0,05
< $15x10^6$ / ml	6,53 %	41,66%	< 0,05
Mobility			
< 50%	26,37 %	25,0 %	> 0,05
> 50%	73,63 %	75,0 %	> 0,05
Viability			
< 50%	32,6 %	58,3 %	< 0,05
> 50%	67,4 %	41,7 %	< 0,05
Morphology			
< 4%	*5,4 %*	*8,3*	< 0,05
> 4%	*94,6 %*	*91,7*	< 0,05
Zinc content			
< 100 mg/l	*43,5 %*	*75 %*	< 0,01
> 100 mg/l	*56,5 %*	*25*	< 0,01

Table 4 shows the differences between groups with different cadmium content in concentration, motility, sperm morphology and zinc content. These differences appear when the cadmium content is more than 10 ng/ml. Fortunately, among the examined, such cadmium content was detected only among 12 people. For the rest parameters of the spermogram, the differences between the groups appeared to be within a chaotic spread and had no significance according to χ^2 Pearson.

After the treatment

Table 4 shows that a significant percentage of examined subjects had a zinc content below 100 mg/L, and additionally, especially high number of patients with a rather low zinc concentration was noticed in high cadmium content group. Pharmacological correction was carried out due to zinc level in seminal fluid, and as the final goal, morphological characteristic of sperm was chosen as it is one of the determining factors of male infertility. After completion of substitution and antioxidant three-month course therapy, changes in cadmium level remained within random fluctuations ($p > 0.05$ according to χ^2 McNemar's criterion), while zinc concentration had increased in both groups.

Table 5

Change of zinc and cadmium content in seminal fluid after three-month course along with zinc medication in daily dose 40 mg

Concentration of metals in seminal fluid (mg/l)			
		1 group	2 group
Zn	Before treatment	119,25 (51,4 – 366)	91,2 (39,1 – 123,4)
	After treatment	188,3 (94 – 280)	197,3 (88 – 217,6)
Cd	Before treatment	0,0047 (0,0005-0,0099)	0,0178 (0,0106 – 0,0385)
	After treatment	0,0041 (0,0007-0,068)	0,0142 (0,0084 – 0,0211)

Table 6

Distribution of spermograms of infertile men with escretor-toxic infertility (after treatment) *

Spermogram parameteres	% of examined men	
	1 group 96 people	2 group 12 people
Concentration		
> $15x10^6$ / ml	95,83 %	83,33 %
< $15x10^6$ / ml	4,16 %	16,66 %
Mobility		
< 50%	19,79 %	16,67 %
> 50%	80,21 %	83,33 %
Viability		
< 50%	32,29 %	33,3 %
> 50%	67,71 %	66,7 %
Morphology		
< 4%	1,04 %	8,33 %
> 4%	98,95 %	91,67 %

Note: groups are divided by cadmium content before treatment

Table 6 shows that as a result of zinc and selenium therapy, sperm quality parameters and zinc content were brought closer in both groups (differences between groups became unreliable).

Discussion

During meta-analysis done by Sun et al., the interaction between men fertility changes and microelements level in seminal fluid, including Cd and Zn, was studied. It has been found that even a small increase of cadmium level significantly reduces the quality of sperm, but not in such a high extent as a decrease of zinc concentration [23].

Zn is one of the most important microelements in proliferating tissues of the human body due to its exceptional role in the process of genome transcription, as well as antioxidant properties; this naturally determines its crucial importance for spermatogenesis, and its deficiency is an presumptive factor of male infertility. Zn deficiency leads to spermatogenesis damage by deactivating several mechanisms, including reduced synthesis of Zn-dependent proteins and nuclear proteins, which are responsible for regulating fission and control processes, resulting in series of DNA damages and cell apoptosis. Deviations of spermatogenesis and reduced sperm quality in Zn-deficient patients are described in a number of studies [24, 25]. For example, Kothari et al. noticed the reduction of Zn levels in seminal plasma during asthenospermia, oligoastenospermia, teratospermia and azoospermia [26]. Kolagar et al. [27] reported that fertile individuals have significantly higher levels of Zn in seminal plasma compared to infertile subjects. An additional but fundamentally important role of zinc is its participation in the oxidative stress control system by regulating the activity and concentration of zinc-dependent fermints, primarily mentioning the Cu/Zn-dependent superoxide dismutase (SOD) subtype.

Our research confirmed the effect of zinc level in seminal fluid on sperm quality (sperm count, motility, viability and normal morphology). As a result of the pharmacological correction by zinc medication at a dosage of 40 mg/day for 3 months, the cytological characteristics of ejaculate had been improved (assessment of dynamics according to McNemar 's donbass 2 < 0.05).

During our research the cumulative clinical and morphological effect of comprehensive corrective therapy for male infertility had been monitored, including selenium medication at a dosage of 200 μg/day for 3 months: and as a result, significant data was obtained regarding the effectiveness of selected treatment for spermogram quality control.

The increase of cadmium level in seminal fluid can lead to reduced reproductive health among men [28]. In various researches, there is information about decrease of ejaculate quality due to the progressive deterioration of certain sperm profile parameters: sperm viability; mobility; concentration and normal morphology [29, 30]. Pant et al. described significant increase of Cd content in sperm of infertile men adjusted for age, weight and possible bad habits in comparison with population of healthy individuals [31].

We show that in the south of Ukraine, the vast majority (85.18%) of the examined infertile men have cadmium content in semen much lower than 10 ng/g. Nevertheless, 12 people with increased cadmium content in seminal fluid were identified. The excess of cadmium content is connected with abnormally low zinc concentration. As a result of complex replacement therapy with zinc and selenium medications in standard dosages, statistically significant increase of spermogram parameters was revealed (dynamics assessment according to χ^2 McNemar's < 0.05).

Conclusions

1. The main but not the only reason of Zinc deficiency is connected with its insufficient food intake. Zinc deficiency is one of the common factors in male infertility.

2. Reduced zinc content in seminal fluid is inversely correlated with concentration, mobility, viability and normal sperm morphology, forming a direct threat to human reproductive health.

3. The increase of Cadmium level in sperm samples of workers with professional insalubrity is inversely correlated with zinc concentration as well as spermogram parameters.

4. Complex replacement and corrective therapy with zinc, selenium, vitamins A and E medications for 4 months leads to improved cytological characteristics of ejaculate.

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