THE ROLE OF LEAD IN THE ETHIOPATHENESIS OF MALE FERTILITY REDUCTION

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SUMMARY

Background. The problem of preserving and strengthening of the reproductive health of the population in conditions of the industrial regions is becoming one of the key.

Aim. To determine whether blood and semen concentration of lead in fertile and infertile men without occupational exposure to lead is associated with semen quality and reproductive outcome.

Materials and methods. Clinical and hygienic assessment of the reproductive health was carried out in 192 men of the Dnipropetrovsk region, living in the industrial and control, conditionally "clean" city.

Results. In the pathogenesis of male fertility, an important role belongs to the level of lead in biosubstrates, which is 1,2-2,1 times higher in industrial cities as compared to the normative level. Therewith, fertilizing properties of the ejaculate of the fertile men living in the industrial region comply with WHO standards, while in the infertile group various forms of pathology was revealed.

Conclusions. Violations of spermatogenesis can serve as a rapidly-responding and reliable criterion for assessing the adaptation and maladaptation processes of men under the influence of xenobiotics of the environment, in particular lead. In this case, the markers of influence are the total number of sperm in the ejaculate, their concentration, mobility and the number of pathological forms, semen viscosity.

Key words: fertility, ejaculate, lead, exposure, biomonitoring, infertility.

Background. The problem of preserving and strengthening of the reproductive health of the population is becoming one of the key medical and social issues. At the same time infertility is the most important violation of the generative sphere, because today the infertile marriage rate is 10-15% in different populations and in 40-50% of cases the cause of infertility is associated with a violation of male fertility that is potentiated by a large-scale anthropogenic pollution of the environment [1, 2]. Nowadays, the most frequent causes of the male infertility are associated with spermatogenesis [3].

Among the xenobiotics of diverse nature, heavy metals are especially dangerous for the human reproductive system in case of excessive intake, according to the WHO data, and lead in particular, which is a well-known polytropic toxin. An increasing amount of lead in the environmental objects and in nutrition is believed to be related to a poor semen quality. The reproductive effects of lead are complex and appear to involve multiple pathways, but not all of which are fully understood [4].

Aim of the research: to determine whether blood and semen concentration of lead in fertile and infertile men without occupational exposure to lead is associated with semen quality and reproductive outcome.

Materials and methods. Clinical and hygienic assessment of reproductive health of the population was carried out in 192 men of Dnipropetrovs'k region, who sought medical consultation in the Center for Family Planning and Human Reproduction of the CI «Dnipropetrovsk regional perinatal center with full-time department DRA» for the period 2012-2015. All patients have given informed consent to the use of research results for a scientific purpose without the indication of personal data.

According to the research design, initial enrollment was conducted by the questionnairy survey among the male respondents living in the industrial city of Dnipro and the control, conditionally "clean" city of Novomoskovsk, this selection as research objects was based on

the main principle of epidemiological surveillance - the homogeneity of the comparative cities in climatic-geographical data, social-hygienic parameters and level of medical care [5].

After the questionnairy survey, all the patients underwent a series of studies in accordance with the standard protocol [6] to form experimental groups according to the following criteria: age of the examined (20-50 years), duration of residence in one city (more than 5 years), identical socio-cultural and economic conditions of life, the absence of chronic diseases, bad habits or occupational hazards, absence of acute diseases or fever during the previous 4 months, duration of marriage for more than 2 years, presence (fertile) or absence (infertile) of children in this or previous marriage. In the group of infertility patients, men with idiopathic infertility were enrolled by the results of complex clinical and laboratory diagnostics.

As a result of the performed survey, 26 men were excluded from the further research because of chronic general-somatic diseases, acute orchitis or infection of the genital tract, violation of the location and size of testicles, alcohol and tobacco abuse, spermatogenesis disorders not related to the environmental contamination (chromosomal anomalies, trauma or inflammation of the genital organs in anamnesis, etc.). Then four experimental groups were formed: 1 group - fertile men living in the city of Dnipro (62 respondents), 2 group - men with normal fertility living in the control town (37 respondents), 3 group - infertile men living in the city of Dnipro (48 patients), group 4 - infertile men, residents of the control city (19 patients). The age of the subjects was within the range of 20-50 years, which on average made up 31.9±0.9 and 30.4±0.6 years for the infertile and fertile men.

To determine the lead concentration, venous blood samples and ejaculate were selected according to the standard methods. Biological samples were transferred to aliquots, frozen and stored at -20 ° C until analysis. The research was carried out using inversion voltammetry on the device AVA-2 in the sanitary-hygienic laboratory of the SE "Dnipropetrovsk Regional Laboratory Center of the State Sanitary-Epidemiological Service of Ukraine" and in the

laboratory of the NPLC "Center" of the SE DMA. In addition, to study the peculiarities of metals translocation from the blood to the male reproductive organs, the index of penetration through the hemato-testicular barrier (IPB) - the ratio of metal concentration in the ejaculate to its content in the whole blood, conventional units (c.u.) was calculated.

As it is well-known [7], the hormonal status, quantitative and qualitative characteristics of the sperm are the criteria for the full value of the male fertility status. To assess gonadotoxic effects of the environmental ecological factors and quality of sperm, ejaculate of males with varying fertility was selected in accordance with WHO requirements [8]. Samples of the sperm were obtained by masturbation after 2-7 days of sexual abstinence. Thereafter, the liquefaction of the samples was carried out within 30 minutes at 37 ° C. Such spermogram findings as sperm volume, sperm concentration, its mobility, morphology and percentage of living forms were assessed using light-optic microscopy. Pathological forms of spermatozoa were classified using "rigid" Kruger criteria [8]. Motility of the sperm was assessed in 60 min. after obtaining the material by classes [6]: A - fast movements; B - slow movements; C - flactuating, pendulum-like movements in place, speed less than 5 microns/sec; D - immovable sperm. The analysis was performed according to criterion-significant fractions: progressively mobile sperm, category A + B; generally mobile sperm, category A + B + C.

In statistical analysis of the results, relative and averaged values were calculated, the normality of the distribution of quantitative indicators was checked; estimation of the reliability of the average difference for quantitative attributes with normal distribution according to Student's criterion (t) was made. Correlation analysis with the calculation of the Spirman rank correlation coefficients (p), the linear Pearson correlation (r); regression analysis with the calculation of multiple (R) and partial correlation coefficients (r) were made. Statistical processing of the results was carried out using statistical software packages of statistical analysis Statistica v.6.1 (StatsoftInc., США, licence N AJAR909E415822FA), Microsoft Excel.

Results. As a result of the conducted research, it was found that the average concentration of lead in the blood of the fertile men of the industrial city was 0.063±0.005 mg/l, control – 0.051±0.003 mg/l, in both cities – 0.059±0.004 mg/l. The content of metal in the semen of the fertile men ranges from 0.02 to 0.100 mg/l, which, on average, is 0.049±0.002 mg/l, with practically identical indices in the industrial and control cities. Concentration of lead in the blood and ejaculate of the infertile men of both cities under the observation practically does not differ and averages 0.061±0.005 and 0.062±0.005 mg/l, respectively. Therewith, the IPB was 0.83 c.u.

Table 1 →

The results of the study of the fertility potential of men in the Dnipropetrovsk region indicate that in all men examined, after 4-7 days of sexual abstinence, the semen volume varied within 2-6 ml, with a specific smell and gray-milky or milky-white color. The viscosity of semen in men with normal fertility varied within 0-0.5 cm and, on average was 0.34±0.02 and 0.23±0.02 cm in the industrial and control cities respectively. In the men of the infertile group, the semen viscosity had significantly greater fluctuation boundaries – 0-10 cm, which, on average was 3.37±0.31 cm and 2.95±0.58 cm in the industrial and control cities respectively.

Table 2 →

The time of the ejaculate liquefaction in fertile men was within 20-40 minutes, infertile ones – 20-60 minutes, the pH reaction was weakly alkaline and averaged 7.49±0.03 – 7.79±0.006 conventional units at fluctuations of this indicator within 7,0-8,0.

The total amount and concentration of sperm in the ejaculate of the fertile men was 90.14±5.58 on average and 96.09±6.83 million, 25.61±1.01 and 28.53±1.11 million/ml in industrial and control cities respectively. In the infertile men of the industrial city the average sperm concentration in the ejaculate was 14.39±1.41 million/ml.

In the males with normal fertility, the progressive sperm motility (category A + B) was 63.39±0.61 and 62.63±0.68% in industrial and control cities respectively. A similar situation was observed with respect to the generally-moving spermatozoa (category A + B + C) –

82.13±0.77 and 81.19±0.64% respectively. In the group of men with idiopathic infertility, the number of progressively-moving spermatozoa (cat. A + B) on average was 32.45±1.81% and 43.3±2.3%, and generally-moving (cat. A + B + C) – 41.87±2.3% and 53.6±3.5% in industrial and control cities respectively.

The number of live forms of sperm in the fertile men of both cities under observation ranged from 51-92% and averaged 72.15-75.28%. In the group of infertile men, the number of live spermatozoa in the ejaculate varied from 0 to 96% and averaged 64.13-73.47%.

The number of forms with normal morphology in all experimental groups ranged from 14-80%, which was 57.96-59.19% on average for fertile males and 50.6-54.7% for infertile ones. No man was found with a decrease of this indicator below the regulatory level – 4%. Pathologically modified forms of spermatozoa occur with the frequency from 40.8±1.1% in fertile men in the control city, to 49.1±2.4% in the infertile men, residents of the industrial city.

Discussion. The results obtained revealed the excess of the normative level of lead content in the blood by 1.2-2.0 times ($p<0,01$) [5] in the men with different fertility; this confirms technogenicity of its origin in the organism. The latter was confirmed while comparing the results of men living in the industrial and control cities of the region - the concentration of lead in the blood of the inhabitants of Dnipro city was 1.2 times higher ($p<0.05$) as compared with the surveyed men of the conditionally "clean" city.

The content of this abiotic metal in the sperm of the fertile men in the Dnipropetrovsk region, similar to the results of the study of metal in the blood, was 1.7 and 2.1 times higher ($p<0.001$) than the normative data [9]. Therewith, the concentration of metal in the blood of the fertile men was 1.2 times higher ($p<0.05$) than its content in the ejaculate, while the difference in the group of infertility men is absent. It should be noted that the concentration of lead in the sperm of infertile men is 1,3 times ($p<0,05$) higher as compared with the values of the fertile group. The revealed differences in the toxicant levels in the biosubstrates of the

fertile and infertile men coincide with the results of other studies [10, 11] and testify to an active accumulation of lead in the ejaculate, which probably leads to the violation of the fertile properties of the sperm and, along with other factors, may be a risk factor for infertility development. The latter assumption is confirmed by the high value of IPB, which indicates that the overwhelming majority of lead penetrates from the blood to the testicles and can lead to a direct damage to the hemato-testicular barrier (HTB) and functional activity of the testicular tissue [12], and, according to data [13] indirectly affect the hypothalamic-pituitary system, raising the level of entropy in the morphofunctional system of the testicles due to the combined action of intravascular (slow blood flow, change in blood rheology), intrmural (damage to the epithelium and serous cells, damage to the integrity of the basement membrane) and extravascular factors (perivascular fibrosis).

Comparison of the ejaculate quality results in fertile and infertile men of both cities under observation revealed that such spermogram findings as the total number of spermatozoa and their concentration, the number of mobile spermatozoa and forms with the normal morphology in the fertile group were 1.1-1.9 times ($p<0,05$ – $p<0,001$) higher, and viscosity and time of liquefaction - by 1,1-12,8 times ($p<0,001$) lower as compared with infertile group. It should be noted that in all fertile men examined, the pH value corresponded to the normative level, while in 32.1% of the infertile men of the industrial city and in 26.7% of the infertile ones of the control city this indicator was lower than the criterion level [13]. Therewith, in the men of the control city, the semen volume complied with the norm in all cases, while in the industrial city this finding was lower than the regulatory level - 39 mln in 7.7% of the patients surveyed.

It was found that in the sterile men the total sperm concentration in the ejaculate is by 4.1% lower the norm and is characterized as oligozoospermia. Therewith, in 42% of infertile men of the industrial city and in 13.3% of the control city absence of sperm in the ejaculate,

i.e. azoospermia was revealed, in 21.1% and 26.7% respectively oligozoospermia was detected. It should be noted that both the total number of spermatozoa in the ejaculate of fertile men and their concentration are 1,6-1,9 times ($p<0,01$) higher as compared with the infertile group.

The analysis of individual characteristics revealed a decrease in the sperm motility, i.e. astenozoospermia, by the fraction of progressively mobile spermatozoa (cat A + B) in 36.4% of the infertile men of the industrial city and in 26.7% of the surveyed ones of the control city, of all-moving sperm (cat A + B + C) - in 27.3% and in 13.7% of men respectively. It should be noted that the number of progressively mobile and generally mobile sperm in the fertile men of both cities is 1.4-1.8 times higher ($p<0.001$) than that of the infertility group.

In the group of the infertile men the number of live sperm forms in the ejaculate by the analysis of individual values revealed that in 21.9% and 13.7% of the patients, a decrease in the number of live sperm in the ejaculate forms, i.e necrospermia was present.

Abnormal sperm forms occur with a frequency from 40.8±1.1% in the fertile men of the control city to 49.1±2.4% - in the those of living in the industrial city, with significantly higher rates in this research group ($p<0.05$). Therewith, in all groups surveyed the pathology of the balanus ranks first – 25.9-31.0%, the second – that of the cervix – 8.47-11.01%, the third - the pathology of the spermatozoid tail part- 4,59-7,09%.

Having grouped all the detected changes in spermograms, we found that the ejaculate in the fertile men of the studied cities is characterized by normosospermia. It should be noted that in the residents of the industrial city the ejaculate quality indices were mostly at the lower level of the norm and were significantly worse than in the residents of the control town, especially concerning viscosity, sperm concentration and number of the live forms – 6.0-32.4% ($p <0.01$). Therewith in the sterile men normozoospermia was not detected in any patient surveyed. Ranking pathological changes of ejaculate of the infertile men in the city of

Table 3 →

Dnipro, in terms of frequency azoospermia ranks first, the second – asthenozoospermia, the third – necrosispermia. At the same time, the distribution of the specific gravity of pathological changes in the ejaculate in the control city revealed a slightly different picture: oligozoospermia ranks first, and the second – astenozoospermia and the third is oligoasthenozoospermia. It should be noted that the ejaculate parameters were better in men of the control city, although the probable differences are characteristic only for the indicators of progressively mobile and generally mobile forms, as well as the number of spermatozoa in the ejaculate – by 1.1-1.3 times ($p<0,05$ – $r<0.01$), which coincides with similar studies [1,14].

Thus, the quality of ejaculate in the fertile men meets the standards [13], while in the infertility group, various forms of the pathology of spermograms have been identified. It should be noted that today there are ongoing discussions among scholars [15, 16] regarding the adequacy of the existing reference values of spermograms in accordance with the latest WHO recommendations, since they are significantly understated and do not fully characterize the fertilizing properties of the ejaculate.

Table 4 →

Data of the complex clinical and hygienic research of the reproductive health of men of Dnipropetrovsk region are largely supplemented by the results of correlation and regression analysis. It was found that lead contained in male biosubstrates affects the quality of semen – increase of the level of lead in blood and ejaculate leads to the increase of the pathological forms of spermatozoa in the ejaculate ($r = 0.75$; $p<0.05$), the increase of the sperm viscosity and the number of pathological forms of spermatozoa ($r = 0.95$-0.98; $p<0.01$), the decrease in the total number of spermatozoa, their concentration and mobility ($r = -0.94$ – -0.96; $p<0.01$); this ties up with the data of other authors regarding the negative influence on spermatogenesis with deterioration of ejaculate quality in men with concentration of lead in the blood at a level higher than 40 mg/dl [4, 5].

We have developed single-factor regression models of the effect of lead content in male biological substrates on spermatogenesis which can be used for diagnosis and prediction of fertility potential of men based on the data of biomonitoring of metal in the blood and ejaculate. Therewith, the threshold, diagnostically significant concentration of lead, according to our data, is 0.025 mg/l (blood) and 0.003 μg/l (ejaculate), which is somewhat lower than in other authors who indicate that a threshold for adverse reproductive effects in men may be in the blood lead range of about 30 to 40μg/dl [10] or more than 0.049 mg/l [5]. Increase of the content of these xenobiotics in different biosubstratates above the threshold level leads to a possible deterioration of reproductive potential of men.

Conclusions.

1. The content of lead in the blood and semen of men of the industrial city is 1.2-2.1 times higher than the normative level and the results of the control city, which confirms the anthropogenic origin of its origin in the body. Therewith, the concentration of metal in the blood of fertile men was 1.2 times higher than its content in the ejaculate, while the difference in the group of infertile men is absent.

2. Fertilizing properties of ejaculate of the fertilite men of the industrial region comply with the WHO standards, while in the infertile group there were revealed different forms of pathology, among which the predominant are azoospermia, asthenozoospermia, oligozoospermia. The quality of ejaculate in the fertile men of the industrial city is 1.1-1.3 times lower than in the control group and 1.1-12.8 times higher than in the infertile group.

3. Violations of spermatogenesis can serve as a rapidly reacting and reliable criterion for assessing the adaptation and maladaptation processes of men under the influence of xenobiotics of the environment. In this case, the markers of influence are the total number of sperm in the ejaculate, their concentration, mobility and the number of pathological forms, the semen viscosity.

4. It has been proved that in the pathogenesis of fertility disorders in men an important role belongs to lead, which causes deterioration of spermatogenesis. The obtained results became the basis for the development of mathematical models for predicting fertile potential of men by the concentration of metals in biosubstrates. In this case the concentration of lead in the blood at the level of 0.025 mg/l, in ejaculate - at the level of 0.003 mg/l are physiologically significant for the reproductive system of men.

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