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# Determination of Extracellular Nucleic Acids in Men of Different Reproductive Age Living in the Aral Sea Region

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**Abstract:** Ecological trouble in the Aral crisis is affecting the health of the population, resulting in an assessment of the reproductive function of men at the molecular-cellular level. *The* aim of the study was to investigate the reproductive health of men living in the Aral Sea region. The object of study was ejaculate of men. In men's ejaculate was determined, the contamination of acid-soluble fractions (ASF), extracellular nucleic acids (RNA,DNA), according to method of L.Markusheva. *The* studies revealed a change in the content of ASF (Acid-soluble fractions), extracellular RNA and DNA in the sperm of men, which can be explained by the influence of negative factors in the complex of ecological disaster zone.

**Key words:**Environmental Crisis · Acid-Soluble Fractions (ASF) · Extracellular DNA · Extracellular RNA · Ejaculate · Sperm

### INTRODUCTION

The Aral Sea crisis is recognized as one of the global environmental problems of today. Extreme ecological situation in the Aral crisis caused a massive chemical pollution of the territory for several decades with high doses of pesticides, herbicides; discharge of industrial wastes into the rivers that feed the Aral Sea. Due to drying of the Aral Sea occurred arid areas, climate change and soil salinity, water supply violation [1]. Prevailing ecological trouble in the region is reflected in the health of the population, which leads to disruption of metabolic processes in the body leading to the development of pathologies. Installed on the endocrine system, immune status, but remains practically not studied the state of male reproductive status in the region, which determined the relevance of our research [2].

To assess the state of the reproductive function of men, our study were carried out on molecular-cellular of acid-soluble fractions (ASF), extracellular nucleic acids (RNA, DNA) in the ejaculate of men in the zone of ecological disaster Kyzylorda region. Until now it was thought that DNA is only in cell structures: mainly in the nuclei of cells and some - in the mitochondria, where it plays the role of a carrier of genetic information. It has

been found that small amounts of DNA outside the cells and found primarily in the blood plasma in animals and humans. It was found that the increase of extracellular nucleic acids amount, may indicate of highly increasing number of diseases that may be taken into account as an early sign of relevant pathologies. This gave a very clear practical significance for further study of circulating nucleic acids.

One simple explanation for the appearance of extracellular nucleic acids in biological fluids can be constantly running in the body processes of cell death and degradation of chromatin. Thus, the first source of extracellular DNA in biological fluids can be necrosis or apoptosis of nucleated blood cell elements and endothelial cells [3, 4].

Interest in the extracellular DNA in biological fluids currently grows more and more, due to the prognostic and diagnostic value of this parameter at ray irradiation, cancer, autoimmune diseases, neurological disorders and post traumatically syndrome. When listed pathology significantly change not only the concentration, but also the fractional composition of the extracellular DNA appearance in biological fluids of its low molecular weight fragments often occurs during cancer, stroke, in patients with ischemic processes [5, 6].

Table 1: Indexes of extracellular nucleic acids in men's sperm,  $(M \pm m)$ 

	Age	ASF, standard.units	RNA, standard.units	DNA, standard.units
Zone of environmental catastrophe	18-29 years	0,72±0,13	0,73±0,12	0,49±0,058
(village Aiteke -Bi,city Aralsk)	30-39 years	$0,68\pm0,087$	$0.88\pm0.082$	$0,57\pm0,04$
	40-49 years	$0,63\pm0,09$	$0,76\pm0,094$	$0,46\pm0,063$
Zone of environmental crisis	18-29 years	0,58±0,4	3,13±2,61**	0,42±0,034*
(villages Zhusaly, Zhalagash, Shiely)	30-39 years	$0,63\pm0,05^*$	0,7±0,053**	$0,56\pm0,054$
	40-49 years	$0,46\pm0,06^*$	$0,6\pm0,048$	$0,69\pm0,09^*$

Note: - reliability of in comparison with the groups of different ecological zones, p<0,001, \*\* - reliability of in comparison with the groups of different ecological zones, p<0,011\*

In view of the above, the purpose of the study was to investigate the extracellular nucleic acids in the semen of men in the zone of ecological disaster Kyzylorda region.

#### MATERIALS AND METHODS

The study involved 486 men of reproductive age in the zone of ecological disaster and 524 men of reproductive age in the area of environmental crisis, which were divided into three age groups. The content of acid-soluble fraction (ASF), representing the substance of a nucleotide nature pool (free nucleotides, oligonucleotides, etc..) and the level of RNA and DNA was determined by the method of Markusheva [7]. As an example was taken amount of the blood of examined people: To 0.5 ml of the blood add 2.55 ml of 0.5 n HclO<sub>4</sub>, mix together and centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is soluble fraction of acids, which contains free nucleotides, oligonucleotides and other substances of nucleotide nature) in test tube. After that add to supernatant 5 ml 10% HClO<sub>4</sub>, mix and leave in thermostat for 10 minutes under temperature of 37 °C, centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is hydrolyzed of RNA) in test tube. Add to supernatant 3 ml of 10% HclO<sub>4</sub>, mix and leave in water bath for 7 minutes under temperature of 70°C, centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is hydrolyzed of DNA) in test tube. Spectrophotometry of soluble fraction of acids, DNA and RNA hydrolyzed realize with spectrophotometer 26 under wavelength 290 nm [7].

Statistical handling of getting data had been provided by parametrical methods with estimation of the difference by t – Student criteria [8].

#### RESULTS AND DISCUSSION

The results of extracellular DNA in the sperm of affected men are demonstrated in Table 1.

Metabolic processes is a violation of all the synthetic and proliferative processes that directly affect the nuclear structure of cells. In this study, we carried out determination of ASF, RNA and DNA in semen as a change in the generation of reactive oxygen species (ROS) can influence the supramolecular complexes and nucleic acids. Significant role in the process of DNA damage may play products of lipid peroxidation (LPO).

The amount of ASF in the men sperm that live in the zone of ecological disaster was higher than the values of indicators in men living in the area of environmental crisis and this trend is observed in all age groups.

## CONCLUSIONS

According to the study of circulating extracellular RNA and DNA in the sperm of men recorded their decline with a corresponding increase of acid precursors, which can be attributed to the degradation of the nucleic acids under the influence of negative factors in the complex area of ecological disadvantage, that is agrees with previously carried out research studies provided by Muravleva L.E. [9] and Tamkovich S.N. [6].

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