

# Features of Anti-radical Activity and Antioxidant Sperm Status in Men Living in the Western Urals

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## ABSTRACT

Inflammatory diseases in the urogenital tract of men living in different climatic conditions often cause infertility. The study aims to assess the antioxidant status and antiradical activity of sperm in men with chronic prostatitis living in the Western Urals. 55 men aged 23 to 38 years with inflammatory diseases of the prostate gland living in the Western Urals were examined. Group 1 consisted of 25 men with chronic prostatitis in remission, group 2 was composed of 30 people with chronic prostatitis at the acute stage. The level of total antiradical activity of sperm (TAAS) was studied using the SF PE-5300 (Russia), the activity of superoxide dismutase (SOD) was studied on the enzyme immunoassay analyzer Sunrise (Tecan, Austria). Results are presented as arithmetic mean (M), standard error (m) and 95 % confidence interval for mean (95 % CI). To test the null hypotheses about the equality of mean values between two independent groups with a normal distribution, the two-sample Student's test was used. The significance level at which the null hypotheses were tested was 0.05. According to the assessment of the antioxidant status of sperm, there was a statistically significant ( $p = 0.039$ ) 20 % increase in the total antiradical activity of sperm in patients with an acute inflammatory process in the prostate gland compared to the similar indicator identified in men without symptoms of the acute inflammation. In the group of patients with acute chronic prostatitis, the share of samples with a significant increase in the level of total antiradical activity of seminal plasma compared to the reference level was 33.3 % versus 23.3 % in men with chronic prostatitis during the remission; the frequency of excess was 1.5 times. The evaluation of sperm parameters showed that the activity of superoxide dismutase in both groups was within the reference range. In the seminal plasma of men with chronic prostatitis during an exacerbation, in 8.8 % of samples, the activity of superoxide dismutase was lower, and in patients with the chronic process in the prostate gland during the remission, the activity of superoxide dismutase was below the reference level in 3.3 % of the samples, the frequency of a decrease was 2.7. Thus, with chronic prostatitis, the imbalance in the oxidation-antioxidation system plays a key role in the development of impaired spermatogenesis, and markers of redox processes can serve as informative indicators of the

**Keywords:** anti-radical activity, antioxidant sperm status, urogenital tract, inflammatory diseases, Western Urals, climatic conditions

## 1. INTRODUCTION

It is known that reactive oxygen species (ROS) play the most significant role in the regulation of the vital activity of the organism and the functional activity of the cell in various climatic and geographical conditions [1, 2]. Of particular relevance is an assessment of the

universal protective and adaptive reactions of the multicellular organism to mechanical, physical, chemical, biological and other environmental factors in order to localize and / or eliminate damaging agents, as well as to restore (or replace) damaged tissues [3]. Researchers argue about the participation of reactive oxygen species (ROS), generated in the inflammation

focus, in redox-regulated signalling cascades in the target cells of the body. Physiological or pathological effects of the ROS are realized depending on the factor influencing the cell, time and intensity of the factor. The result of ROS-mediated signalling is a change in the level of gene expression, activation of nuclear transcription factors, initiation or inhibition of apoptosis. It has been proven that ROS overproduction can damage cells and tissues and contribute to the chronic inflammation underlying metabolic and neurodegenerative disorders, cardiovascular pathology, and respiratory system diseases in men in various climatic conditions [1–4].

The main sources of reactive oxygen species in semen are leukocytes and sperm cells. It is the ROS level that regulates the spermatogenesis [4]. The participation of reactive oxygen species in the condensation of chromatin in spermatozoa has been proven, and the role of ROS in the regulation of proliferation and apoptosis of spermatogonia has been established [5]. Various intracellular and extracellular stimuli can provoke excessive accumulation of reactive oxygen species and lead to detrimental effects on sperm function, in particular, damage to sperm DNA, which reduces the likelihood of pregnancy [6]. The antioxidant system consisting of enzymatic and non-enzymatic links protects the cell from the negative consequences of ROS. It is relevant to assess the antioxidant status of sperm plasma in men with inflammatory processes in the prostate gland, living in different climatic conditions.

The aim is to evaluate the antioxidant status and antiradical activity of sperm in men with chronic prostatitis living in the Western Urals.

**2. MATERIALS AND METHODS**

The study was conducted in compliance with the ethical requirements fixed in the 2000 Helsinki Declaration by the WMA and the Protocol of the Council of Europe Convention on Human Rights and Biomedicine (1999). In total, 55 men aged 23 to 38 years with inflammatory diseases of the prostate gland living in the Western Urals were examined. Group 1

consisted of 25 people with chronic prostatitis in remission, group 2 – of 30 men with acute chronic prostatitis.

The level of total antiradical activity of sperm (TAAS) was studied using the SF PE-5300 (Russia); the activity of superoxide dismutase (SOD) was studied with the enzyme immunoassay analyzer Sunrise (Tecan, Austria).

The distribution of quantitative data was checked using the Kolmogorov-Smirnov statistical test. The arithmetic mean (M), standard error (m), and 95 % confidence interval for the mean (95 % CI) were used to describe data that were normally distributed. To test the null hypotheses about the equality of mean values between two independent groups with a normal distribution, the two-sample Student's t-test was used. The significance level at which the null hypotheses were tested was 0.05. Statistical analysis of the data was carried out in Statistica 6.0 (StatSoft, USA)

**3. RESULTS**

It was found that the average value of the total antiradical activity of sperm plasma in the group of men with chronic prostatitis without exacerbation corresponded to the norm (Table 1). The study of sperm quality revealed a statistically significant ( $p = 0.039$ ) 1.2-fold increase in the TAAAS level in men with acute chronic prostatitis relative to the values obtained in men without symptoms of the acute inflammatory process. Evaluation of sperm parameters showed that the activity of superoxide dismutase in both groups was within the reference values.

The TAAS of seminal plasma in group 2 (exacerbation stage) varied from 207 to 3010  $\mu\text{M/L}$ , and in group 1 (without exacerbation) it varied from 40 to 1570  $\mu\text{M/L}$  (Table 2). In men with an acute inflammatory process (group 2), the share of samples with a significant increase in the level of total antiradical activity of seminal plasma and activity of superoxide dismutase was 33.3 and 28.8 versus 23.3 and 16.6 in men with chronic prostatitis during the remission (group 1); the frequency of an excess was 1.6 times.

**Table 1.** Indicators of antioxidant activity of spermoplasma in men, m (m); 95 % CI

Indicator	Reference level	Group 1 $n=25$	Group 2 $n=30$	$t$	$p$
TAAS, $\mu\text{M/L}$	500–1000	794.88 (47.40) 755.68–834.08	960.69 (62.92) 889.39–1032.53	2.11	0.039
SOD, U/ml	164–270	245.34 (56.25) 224.34–266.35	242.41 (65.93) 222.60–262.21	0.030	0.973

The share of sperm plasma samples with a reduced level of TAAS in group 2 (exacerbation stage) was

17.7 % versus 26.6 % in group 1 (without exacerbation), the frequency of a decrease was 1.5 times. In the

seminal plasma of men with chronic prostatitis during the exacerbation (group 2) in 8.8 % of the samples, the SOD activity was below the standard values, while in 3.3 % of the samples of men with a chronic process in

the prostate gland during the remission (group 1), the superoxide dismutase activity was below the reference value; the frequency of a reduction was 2.7 times.

**Table 2.** The frequency of registration of samples of antioxidant status indicators with deviations from reference values, %

Indicator	Group 1 <i>n</i> =25			Group 2 <i>n</i> =30		
	Range of values	Abnormal samples		Range of values	Abnormal samples	
		higher	lower		higher	lower
TAAS, μM/L	40–1570	23.3	26.6	207–3010	33.3	17.7
SOD, U/ml	154.21–398.54	16.6	3.3	114.85–444.43	28.8	8.8

An increase in the total antiradical activity of sperm plasma during the active inflammatory process suggests an excessive generation of reactive oxygen species against the background of the oxidative stress in sperm. Under the physiological conditions, various non-enzymatic and enzymatic systems are responsible for redox homeostasis; however, the imbalance between the prooxidant and antioxidant systems during inflammation causes metabolic disorders and energy supply of germ cells. Obviously, oxidative stress in sperm can cause male infertility.

It has been proven that an increased level of reactive oxygen species in inflammatory diseases of the urogenital tract is due to the unsatisfactory sperm profile, characterized by a high share of inactive mitochondria, abnormal expression of mitochondrial proteins, and changes in the calcium signaling cascade [7–10]. The altered level of calcium in the sperm was due to the low activity of Na<sup>+</sup> / K<sup>+</sup> -ATPase of the sperm, which reduces the mobility of the male reproductive cell [11]. Changes in the functional activity of mitochondria can cause impaired sperm motility [12]. The participation of ROS in the dephosphorylation of glycogen synthase kinase 3α (GSK), a negative regulator of sperm motility, has been suggested [13]. The significant effect of glycogen synthase-3β kinase on the opening / closing of the mitochondrial pore has been proven [1–4].

We should note that reactive oxygen species induce multiple cellular physiological processes (proliferation, differentiation, apoptosis, etc.) in physiological conditions [14,15]. It is interesting that sperm cells with defective morphology turn out to generate reactive oxygen species in greater amounts, for example, as a response to inflammation or negative exposure to exogenous factors, than sperm cells with proper structure. In case there is acute inflammation, ROS are generated in significant quantities and it results, in particular, in Nrf2 / ARE activation and induction of some enzymes [16]. Nrf2 / ARE activation increases activity of superoxide dismutase and glutathione

peroxidase (antioxidants), certain enzymes in P-450 cytochrome family (enzymes participating in I detoxification phase), glutathione transferase (enzymes participating in II detoxification phase) [17]. ROS [18, 19] and glutathione [20] are assumed to participate in a chain for transferring signals that activated SOD and expression of its genes at transcription level. Superoxide dismutase deficiency in a body results in weaker protective functions and damage to cells done by free radicals. Meanwhile increased SOD activity is able to induce excessive H<sub>2</sub>O<sub>2</sub> quantities in a body and, consequently, result in toxic damage to cells [21].

Lipid peroxidation that occurs mostly due to effects produced by reactive oxygen species makes for changes in expression of surface membrane receptors and also can induce disorders in activity of matrix enzymes in mitochondria and dysfunction of components in the respiratory chain. It has been shown on many cellular models that increased ROS generation can induce cell death and internal bio-energetic reserve is a basic factor that determines cellular response; it has also been shown that a cell should be provided with sufficient energy for starting detoxification and apoptosis. In case there is irreversible mitochondria dysfunction and energy crisis, “string lethal” stimuli induce cellular necrosis. Obviously, oxidative stress induces transcription of specific genes, activates the most significant enzymes, modulates intracellular signal pathways that coordinate cell life cycle; given all that it can modify the most significant functions performed by gamete changing its vital activity depending on duration and extent of oxidation-recovery imbalance [22–25].

Quantitative and qualitative properties of human sperm tend to deteriorate and it has been detected worldwide recently together with growing number of dysfunctions in the reproductive system; it is a serious medical problem that seems only to aggravate thus overstepping medical boundaries and becoming a social and demographic issue. Given that, it is truly vital to examine reasons for male infertility including studies performed on men living in different climatic and

geographic conditions. According to some authors, oxidative stress which causes male infertility in 30–80 % cases [26] also plays a key role in DNA fragmentation [27], lower sperm cells mobility [28], and male gamete death [29]. Reproductive health is a significant component in men's overall health and welfare. Improving and preserving reproductive potential allow avoiding sexual diseases and provide efficient preservation and growth of male fertility.

### 3. RESULTS

Thus, the analysis of the antioxidant status of sperm plasma in men with chronic prostatitis living in the Western Urals determined its specific features taking into account the inflammatory process. It was found that in men with chronic prostatitis at the decompensation stage, a statistically significant ( $p = 0.039$ ) 1.2-fold decrease in the TAAS level of sperm was observed

The results indicate a high potential antiradical activity of sperm plasma in men with inflammatory diseases, which may indicate an excessive increase in the production of free radicals during the exacerbation. The TAAS changes in sperm plasma in men with chronic diseases of the urogenital tract play a pathogenetic role in the development of spermatogenesis disorders, and markers of redox processes can serve as informative indicators of the inflammatory process in men living in the Western Urals.

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