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The Processes of Lipoperoxidation and Antioxidant Protection in Men with Different Variants of Spermograms

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Abstract

Background: Currently, infertile couples represent a very complex and serious medical and social problem. In 40%-60% of cases, the reason for the absence of children in the family is the male factor. Oxidative stress (OS) has been identified as one of the many mediators of male infertility by causing sperm dysfunction. The purpose of this study was to identify the characteristics of the intensity indices of lipid peroxidation (LPO) and antioxidant system (AOS) in the ejaculate of men with different spermogram variants.

Materials and Methods: We performed a retrospective analysis of the results of examinations of 69 men of infertile couples with various disorders of the ejaculate and 155 healthy men with proven fertility. In the semen of the examined men, the level of CD (conjugated dienes), TBARS (thiobarbituric acid reactive substances), α-tocopherol, retinol and total antioxidant activity (AOA) was evaluated by spectrophotometric method.

Results: We found that the antioxidant protection system in patients with asthenozoospermia was characterized by a decrease in the level of total AOA in the semen by 50% and the α–tocopherol concentration by 52%, compared to healthy men in the control group. In patients with oligozoospermia, an increase in the CD concentration by 39% and a decrease in the TBARS concentration by 26% was found; the level of total AOA decreased by 37% and the concentration of α–tocopherol by 41%, compared to healthy men.

Conclusion: In general, the analysis of the obtained data of the survey of men with different variants of spermograms indicates a change in the parameters of the LPO–AOS and confirms the OS development. Thus, it can be noted that depending on the pathological state of the ejaculate in men of reproductive age, LPO processes have their own characteristics, and in men with oligozoospermia, LPO processes occur more intensively. Activation of LPO-AOS processes can be both a consequence and a cause of various metabolic changes in the human body. (**International Journal of Biomedicine. 2019;9(2):168-171.**)

Key Words: men • sperm • lipid peroxidation • antioxidants

Abbreviations

AOA, antioxidant activity; **AOS,** antioxidant system; **CD**, conjugated dienes; **ROS**, reactive oxygen species; **OS**, oxidative stress; **LPO**, lipid peroxidation; **TBARS**, thiobarbituric acid reactive substances.

Introduction

Currently, infertile couples represent a very complex and serious medical and social problem.(1-4) Despite the improvement of clinical-laboratory examinations and methods, and the introduction of auxiliary reproductive technologies into the wide clinical practice, the incidence of infertility in the marriage varies widely (7-28%) and does not tend to decrease.(5) In 40%-60% of cases, the reason for the absence of children in the family is the male factor.(4-7) According to the literature, many factors affect the quality of the ejaculate, including an unfavorable ecological situation, inadequate and unbalanced nutrition, smoking, alcohol, inflammatory diseases of the genitourinary system, varicocele, and food products.(1,4)

OS has been identified as one of the many mediators of male infertility by causing sperm dysfunction. Active forms

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of oxygen (ROS) are necessary; under normal physiological conditions, they contribute to the reaction of capitation, regulation of maturation of spermatozoa and the development of cellular signaling pathways. Higher levels of ROS induce LPO, damage to sperm DNA and apoptosis.[8] To overcome these undesirable consequences, ROS are naturally stabilized by the components of the body's antioxidant defense. In a healthy body, pro-oxidants and antioxidants remain in balance. However, under pathological conditions, the uncontrolled production of ROS exceeds the antioxidant capacity of the seminal plasma, resulting in OS.[1,9-12] Spermatozoa are particularly vulnerable to OS because they do not have the necessary cytoplasmic antioxidant recovery systems. 20%-40% of infertile men have a higher level of ROS in the semen than in healthy men.[13]

Lipid composition of cell membranes of spermatozoa affects their functional characteristics.[14] Long-chain polyunsaturated fatty acids in a high concentration are present in the male germ cells. Their number in relation to saturated fatty acids and cholesterol is closely related to the fluidity of the membranes of spermatozoa.[15] Due to a significant number of double bonds, polyunsaturated fatty acids in the membranes of spermatozoa are particularly susceptible to LPO when there is an increase in the total amount of oxygen compounds formed and an imbalance in the components of the antioxidant system. In male infertility, the role of reactive oxygen species and decreased AOA in seminal plasma was established. [2,16]

The purpose of this study was to identify the characteristics of the intensity indices of LPO and AOS in the ejaculate of men with different spermogram variants.

Materials and Methods

We performed a retrospective analysis of the results of examinations of 69 men of infertile couples of Irkutsk city with various disorders of the ejaculate. All patients were divided into two groups: Group 1 included 45 men (mean age of 30.2±3.6 years) with astenozoospermia; Group 2 included 24 men (mean age of 31.9±7.5) with oligozoospermia. The control group consisted of 155 healthy men (mean age of 31.6±5.9 years) with normozoospermia and a realized reproductive function.

Exclusion criteria were obesity, type 1 and type 2 diabetes, arterial hypertension, endocrine infertility, inflammatory diseases of the urogenital tract, including sexually transmitted infections.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki (2000; revised October 2013, Fortaleza, Brazil). Written informed consent was obtained from all participants.

Methods of standard clinical examination of fertile and infertile men included: an ultrasonic scan of scrotum and prostate, macroscopic and microscopic examination of ejaculate, and biochemical analysis. The semen analysis was performed in accordance with the WHO recommendations.[17]

In the semen of the examined men, the content of CD (primary oxidation products) and TBARS, end products of LPO, was determined by the methods of V. Gavrilov et al.[18,19] The level of α-tocopherol and retinol was estimated by the method of R. Ch.Chernyauskene et al.[20] AOA according to GI Klebanov et al.[21] The measurements were performed using a Shimadzu RF-1501 spectrofluorophotometer (Japan).

The statistical analysis was performed using the statistical software STATISTICA 6.1 (StatSoft Inc., USA). The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

OS has been identified as one of the many mediators of male infertility. It is shown that in 30%-80% of cases of male infertility, pathospermia are caused by high levels of ROS in seminal plasma.[2,13,14]

We found that the antioxidant protection system in patients with asthenozoospermia was characterized by a decrease in the level of total AOA in the semen by 50% and the α–tocopherol concentration by 52%, compared to healthy men in the control group (Table 1).

Table 1

Parameters of the LPO-AOS system in healthy men (Control group) and in men with asthenozoospermia (Group 1)

Parameter	Control group	Group 1	P-value
CD (μmol/l)	1.27±0.81	1.31±0.94	0.791
TBARS (μmol/l)	1.06±0.61	0.89±0.46	0.091
AOA (unit)	3.86±2.26	1.94±1.41	0.000
α–tocopherol (μmol/l)	5.27±2.99	2.53±1.90	0.000

As known, α–tocopherol helps to preserve both sperm motility and morphology, protecting the components of sperm membranes from damage by OS.[22] A number of studies have confirmed the positive effect of antioxidants on spermatogenesis disorders caused by OS.[23-25]

In patients with oligozoospermia (Table 2), we found an increase in the CD concentration by 39% and a decrease in the TBARS concentration by 26%; the level of total AOA decreased by 37% and the concentration of α–tocopherol by 41%, compared to healthy men.

Table 2

Parameters of the LPO-AOS system in healthy men (Control group) and in men with oligozoospermia (Group 2)

Parameter	Control group	Group 2	*P*-value
CD (μmol/l)	1.27±0.81	1.77±0.73	0.005
TBARS (μmol/l)	1.06±0.61	0.78±0.29	0.031
AOA (unit)	3.86±2.26	2.44±1.61	0.003
α–tocopherol (μmol/l)	5.27±2.99	3.14±2.10	0.001

The primary products of LPO, as a rule, are very unstable substances and are easily subjected to further transformations with the formation of more stable oxidation components—aldehydes, ketones, low-molecular acids—as a result of which they exhibit a wide range of changes. A decrease in the level of TBARS in men with oligozoospermia may indicate activation of the enzymatic component of antioxidant protection, in particular SOD, and oxidized and reduced glutathione. [1,24,26]

Thus, the low level of total AOA and α-tocopherol concentration in men with oligozoospermia and asthenozoospermia (Tabl. 1,2) indicates the activation of LPO process. Dramatic changes in cellular redox systems trigger the suppression of antioxidative defense in biological tissues and internal environments. Failure of antioxidant protection can lead to the following changes: damage to membranes, inactivation or transformation of enzymes, suppression of cell division, and accumulation of inert polymerization products in cells.[3]

Reduced sperm motility (asthenozoospermia) and low sperm count (oligozoospermia) are significant causes of male reproductive failure. Their origin is diverse and, in some cases, cannot be established. Reduced sperm motility can often be associated with ultrastructural flagellum disorders, which is a consequence of the genetic nature, as well as the result of external factors—adverse environment, smoking, alcohol, poor and micronutrient diet, sedentary lifestyle and much more.[27] OS causes an arrest of spermatogenesis at the early meiotic stage and induces apoptosis, leading to oligozoospermia.[28] Elevated ROS levels lead to the development of DNA mutations and damage to cell structures with the development of teratozoospermia.[7,28] In preventing oxidative stress and reducing its negative impact on spermatogenesis, the simultaneous use of fat- and water-soluble vitamins is promising.[8] In experiments to study the effects of α–tocopherol, an increase in the mobility and functioning of sperm, and in the frequency of fertilization, was established.[29-31]

Conclusion

In general, the analysis of the obtained data of the survey of men with different variants of spermograms indicates a change in the parameters of the LPO–AOS and confirms the OS development. Thus, it can be noted that depending on the pathological state of the ejaculate in men of reproductive age, LPO processes have their own characteristics, and in men with oligozoospermia, LPO processes occur more intensively. Activation of LPO-AOS processes can be both a consequence and a cause of various metabolic changes in the human body.[32]

Competing Interests

The authors declare that they have no competing interests.

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