# ИЗУЧЕНИЕ СОДЕРЖАНИЯ ИММУНОГЛОБУЛИНОВ И ОКИСЛЕННЫХ БЕЛКОВ В ЭЯКУЛЯТЕ ПРИ БЕСПЛОДИИ

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**Резюме.** Среди причин мужского бесплодия достаточно внимания уделяется окислительному стрессу, который в свою очередь является патогенетическим звеном воспалительного процесса. Однако практически нет информации о содержании окисленно модифицированных протеинов в спермальной жидкости, что затрудняет изучение патогенеза заболеваний репродуктивной системы мужчин. Отчасти окисление протеинов может быть обусловлено продукцией активных форм кислорода микроорганизмами как напрямую, так и опосредованно через активацию клеток иммунной системы. Цель исследования – изучить уровень окислено модифицированных белков и изменения концентрации иммуноглобулинов в спермальной жидкости при бактериоспермии. Проведено исследование эякулята 48 мужчин, обратившихся в клинику по поводу бесплодия в браке. Группу сравнения составили 32 практически здоровых мужчины, у которых отсутствовал рост микроорганизмов в образцах эякулята. При проведении бактериологического анализа исследуемые образцы разводили в 10 раз и использовали общепринятую методику. В спермальной жидкости определяли концентрацию альбумина, иммуноглобулинов A, M, G, E. Окислительную модификацию белков оценивали в реакции с 2,4-динитрофенилгидразином. Концентрацию окисленных белков выражали в нмоль/мг общего белка исследуемой биологической жидкости. Для определения концентрации белка использовали биуретовый метод. Статистический анализ результатов проводили с использованием методов описательной статистики и t-критерия Стьюдента для парных данных. Концентрация белка в семенной жидкости среди изучаемых групп существенно не отличалась. Концентрация альбумина (16,96±1,28 мг/мл) была статистически значимо ниже при отсутствии роста микроорганизмов, чем при бактериоспермии. При бактериоспермии отмечено снижение концентрации IgM и IgA и повышение уровня IgG. Степень окисления белков максимальна при выделении из семенной жидкости энтеробактерий. Таким образом, в ходе исследований было установлено, что, несмотря на отсутствие клиники, при бессимптомной бактериоспермии наблюдается секреция иммуноглобулинов G в спермальную жидкость. Показано накопление окисленных белков в семенной жидкости при бактериоспермии.

*Ключевые слова: окисленно модифицированные белки, спермальная жидкость, динитрофенилгидразин, альдегидные производные, кетоновые производные, иммуноглобулины*

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# STUDY OF THE IMMUNOGLOBULIN AND OXIDIZED PROTEIN CONTENT OF SEMEN UNDER INFERTILITY

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**Abstract.** Among the causes of male infertility, enough attention is paid to oxidative stress, which in turn is a pathogenetic link in the inflammatory process. However, there is practically no information on the content of oxidized modified proteins in the semen, which makes it difficult to study the pathogenesis of diseases of the male reproductive system. In part, protein oxidation may be due to the production of reactive oxygen species by microorganisms, both directly and indirectly through the activation of immune system cells. The aim of the research was to study the level of oxidized modified proteins and changes in immunoglobulin concentrations in the semen under bacteriospermia. A study was made of the ejaculate of 48 men who applied to the clinic for infertility in marriage. The comparison group consisted of 32 practically healthy men who had no growth of microorganisms in the ejaculate samples. When conducting bacteriological analysis, the studied samples were diluted 10 times and used the generally accepted method. The concentration of albumin, immunoglobulins A, M, G, E was determined in the spermatic fluid. The oxidative modification of proteins was evaluated in the reaction with 2,4-dinitrophenylhydrazine. The concentration of oxidized proteins was expressed in nmol/mg of the total protein of the studied biological fluid. The biuret method was used to determine the protein concentration. Statistical analysis of the results was performed using descriptive statistics and Student's t-test for paired data. The concentration of protein in the seminal fluid did not differ significantly among the studied groups. The albumin concentration (16.96±1.28 mg/mL) was statistically significantly lower in the absence of microorganism growth than in bacteriospermia. With bacteriospermia, a decrease in the concentration of IgM and IgA and an increase in the level of IgG were noted. The degree of protein oxidation is maximum when enterobacteria are isolated from seminal fluid. Thus, during the studies it was found that, despite the absence of a clinic, with asymptomatic bacteriospermia, the secretion of immunoglobulins G into the semen is observed. The accumulation of oxidized proteins in the seminal fluid in bacteriospermia has been shown.

*Keywords: oxidized proteins, semen, dinitrophenylhydrazine, aldehyde derivatives, ketone derivatives, immunoglobulins*

## Introduction

Numerous research report a decrease in the quality of semen and other markers of male reproductive health [6, 7]. There is a global crisis of male reproductive health [3], which is confirmed by a global decrease in the number of spermatozoa and an increase in anomalies of the male reproductive system, such as cryptorchidism, tumors of the urogenital tract [2]. The male factor of infertility occurs in about 40% of couples suffering from infertility [1, 5]. The data demonstrate a link between male infertility and general health [2].

It has been shown that asymptomatic bacteriospermia is often found in men who are in infertile marriages. It describes both the direct negative effect of microorganisms on the quality of ejaculate, and indirectly through the reactions of the macroorganism [4]. At the same time, humoral factors of acquired immunity are one of the links of protection against infection. The inflammatory response also implements mechanisms of auto-injury, in particular by hyperproduction of activated forms of oxygen and nitrogen, which trigger carbonyl stress and accumulation of oxidized modified proteins (OMP), which lose their function. The determination of the OMP-level in ejaculate can be a sensitive integral marker of a decrease in male fertility, as well as the development of urogenital pathology of both infectious and non-infectious nature.

**The aim of investigation** was to evaluate the content of immunoglobulins M, G, A, E and the intensity of the accumulation of OMP in the sperm fluid of men with asymptomatic bacteriospermia.

## Materials and methods

Investigation of the ejaculate of 48 men who applied to the clinic for infertile marriage was conducted. The comparison group consisted of 32 practically healthy men who had no growth of microorganisms in the ejaculate samples. The collection of the material and its study were carried out according to standardized methods proposed by WHO experts [9].

For bacteriological analysis, the studied samples were diluted tenfold. Bacteriological examination was performed according to the generally accepted method [4]. The total microbial number was expressed in lg of colony-forming units per 1 mL (lg CFU/mL). Albumin concentration was measured using reagent

kits with bromcresol green (Russia). To determine the concentration of immunoglobulins M (IgM), G (IgG), A (IgA) and E (IgE), an enzyme immunoassay was used (Russia). The concentration of Ig and albumin was expressed in mg/g of total protein.

The OMP was evaluated by reaction with 2,4-Dinitrophenylhydrazine [9], with preliminary incubation of the sperm fluid with a 10% solution of Streptomycin Sulfate. The resulting 2,4-dinitrophinylhydrazones (DNP) were recorded at the following wavelengths: at 365 nm for ketone-DNP of a neutral nature (kDNPn), at 432 nm – ketone-DNP of a basic nature (kDNFb) and at 530 nm – aldehyde-DNP of a neutral nature (aDNFn). When determining the carbonyl content, a molar absorption coefficient (e) equal to 22,000 $M^{-1}cm^{-1}$ was used. The molar extinction coefficient of DNP was used to calculate the concentration of carbonyl derivatives of OMP. Optical density was measured using a flatbed spectrophotometer (USA) with a quartz 96-well microplate.

The concentration of oxidized proteins was expressed in μmol/mg of total protein of the studied biological fluid [8]. To determine the protein concentration, a biuretic method was used (Russia). The protein concentration was measured in each sample after complete dissolution of the protein precipitate in urea solution. The total concentration of oxidized proteins was calculated by summing all oxidized derivatives.

Statistical analysis of the results was carried out using descriptive statistics and Student's t-test methods for paired data, correlation analysis was carried out using Spearman's rank correlation coefficient.

## Results and discussion

It was found that in men with bacteriospermia, 67% had gram-positive cocci (group 1; 4.51±0.26 lg CFU/mL), in (33%) – gram-negative bacilli (group 2; 5.38±0.29 lg CFU/mL, $p < 0.05$ to group 1), the 3rd group consisted of a comparison group without bacterial growth.

The protein concentration in the sperm fluid in the 3rd group was 54.5±2.6 mg/mL, which is not statistically significant from concentration in the 1st (59.0±2.7 mg/mL; $p > 0.05$) and 2nd groups (57.3±3.9 mg/mL; $p > 0.05$).

The albumin concentration of 16.96±1.28 mg/mL and its proportion to the total protein of 30.8±1.5% in group 3 is statistically significantly lower ($p < 0.05$) than in group 1 (21.12±1.41 mg/mL and 35.3±1.2%, respectively) and in group 2 (22.56±2.29 mg/mL and 38.6±2.2%, respectively). However, groups 1 and 2 did not differ statistically significantly from each other.

The highest levels of IgM and IgA were discovered in the comparison group. However, in contrast, the minimum level of IgG was recorded in this group (Table 1). Concentrations of different classes of immunoglobulins did not differ statistically significantly between group 1 and 2. Immunoglobulins E were not detected in any sample.

The total concentration of OMP in group 1 was 1252±22 μmol/mg of total protein, and the ratio of oxidized serum proteins aDNPn : kDNPb : kDNPn was expressed as: 1:1.7:3.2; in group 2, the sum of OMP was 1674±64 μmol/mg of total protein, the ratio of OMP fractions: 1:1,8:3,3. In group 3, the amount of OMP was 598±20 μmol/mg of total protein, and the fractions of OMP were correlated as: 1:1.4:2.2.

**TABLE 1. CONTENT OF IMMUNOGLOBULINS IN THE SPERM OF INFERTILE MEN**

| Group | Content of immunoglobulins, mg/g of total protein | | |
|---|---|---|---|
| | IgG | IgM | IgA |
| 1 | 231.8±11.4* | 4.6±0.9* | 34.6±3.5* |
| 2 | 237.6±23.0* | 5.0±0.8* | 30.8±3.8* |
| 3 | 167.2±12.7 | 12.4±2.9 | 96.2±10.0 |

Note. *, $p < 0.05$ when compared with the data of the 3rd group.

**TABLE 2. CONCENTRATION OF DIFFERENT OMP FRACTIONS IN SEMEN**

| Groups | Concentration of OMP, μmol/mg of total protein | | |
|---|---|---|---|
| | Ketone-DNP of a neutral nature | Ketone-DNP of a basic nature | Aldehyde-DNP of a neutral nature |
| 1 | 675±38#,* | 365±20#,* | 212±9#,* |
| 2 | 909±117* | 493±56* | 272±20* |
| 3 | 282±30 | 187±18 | 129±12 |

Note. *, $p < 0.05$ in comparison with the data of the 3rd group; #, $p < 0.05$ between the indicators in groups 1 and 2.

The concentrations of every DNP derivatives are presented in Table 2. It is worth noting that in the group with germination of microorganisms of the Enterobacteriaceae family, the concentrations of all OMP derivatives ($p < 0.05$) significantly prevail compared to the other groups.

Data analysis shows a moderate direct correlation between the concentration of IgG and OMP fractions in the comparison group ($r = 0.41$) and in the 1st group ($r = 0.44$). In group 2, inverse correlation was found between the concentration of IgA and OMP fractions ($r = -0.54$).

The increase of albumin concentrations in groups with bacteriospermia can be explained by the effect of microorganisms on the microcirculation, resulting in increased transudation of such "heavy" molecules, but this mechanism need to study. The elevation IgG levels in the groups from patients with bacteriospermia are explained by chronic bacterial infection in the genitourinary tract, but the decrease of IgA and IgM levels in these groups can be associated with the suppression of the pool of secretory immunoglobulins of both class A and M, which also need to study. In chronic bacterial infection, the spectrum of OMP shifts towards ketone derivatives of DNP, which according to some data indicates the irreversible nature of protein oxidation.

Probably due to a stronger of the opsonizing ability of IgG in comparison with immunoglobulins of other classes, the activation of the cellular link of innate immunity occurs, which leads to “oxidative stress" and the accumulation of OMP. The persistence of gram-negative flora leads to a more active accumulation of OMP, possibly due to a less secretory immunoglobulins level, whose less pronounced opsonizing activity (compared to IgG) does not lead to such active intensification of “oxidative stress".

## Conclusion

Thus, our investigation has shown that, despite the absence of a clinic, with asymptomatic bacteriospermia, the secretion of immunoglobulins G into the sperm is observed, and the level of immunoglobulin A and M is significantly reduced. The accumulation of oxidized proteins in seminal fluid from patients with bacteriospermia is also shown, and OMP accumulates more intensively during gram-negative bacilli contamination.

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