Semen microbiota and cytokines of healthy and infertile men

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In this study, we determined the levels of cytokine secretory inhibitors and the microbiota biofilms of semen from healthy and infertile subjects. A total of 118 clinical bacterial isolates were isolated and tested. Cytokine secretory inhibitors were determined based on the difference in cytokine content between the control and experimental samples of cell-free supernatants of isolated microorganisms. Biofilm formation was studied by determining the adhesion of microorganisms to the surface of a 96-well sterile plate and expressed as the optical density at 630 nm (OD$_{630}$). Cell-free supernatants of Staphylococcus contained higher levels of secretory inhibitor of cytokines in conditionally healthy than in infertile patients. In contrast, in infertile men, the ability to reduce cytokine levels was more characteristic of Enterococcus and Corynebacterium. Seminal Staphylococcus, Corynebacterium, and Enterococcus isolated from infertile subjects showed a greater ability to form biofilms than the same bacteria isolated from healthy men. Further research is needed on this topic, since it is necessary to determine the relationships between decreased secretory inhibitors of cytokines, production of biofilms by bacteria in semen, and infertility. It is likely that the ability of microorganisms to change the concentration of cytokines and increase the level of biofilm formation in semen may be associated with minimal impairments of fertilizing ability, which are not detected using other methods.

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INTRODUCTION

Infertility, a condition different from various existing health-related issues involving psychological and social aspects, affects 10%–15% of couples in reproductive age. Furthermore, infectious etiologies involving bacteria, viruses, fungi, and protozoa contribute to 15% of male factor infertility. Some studies have shown that the presence of bacteria in semen is common, including in healthy people. The seminal plasma has a specific microbiota, and it can be postulated that the presence of a specific bacterial milieu may not be deleterious but necessary for normal sperm function.

It is difficult to establish exactly which microorganisms are involved in the pathogenesis of infertility, but it is extremely important to determine both microbial factors and host factors that contribute to its development, since the commensal microbiota plays a role in the regulation of local immune homeostasis of the biotope.

Cytokines are hormone-like peptides that regulate intercellular interactions of all parts of the immune system. Currently, secretory inhibitors of cytokines are being actively studied, that is, the ability of cell-free supernatants of microorganisms to reduce the level of cytokines after their cocultivation. The important and paramount role of the immune status at the local level in the pathogenesis of chronic prostatitis has also been noted. However, the available research on the immunomodulatory effect of symbiotic bacteria does not allow the effect of microorganisms on the level of cytokines in semen to be assessed. In addition, the study of biofilm formation by microorganisms inhabiting the urethra is of great importance, since this makes it possible for microorganisms to avoid various host antimicrobial immunity proteins, which contribute to the development of infection.

This work aimed to determine the level of a secretory inhibitor of cytokines and biofilm formation by the seminal microbiota of healthy and infertile subjects.

PARTICIPANTS AND METHODS

Participants

The study was carried out at the infertility clinic at Orenburg Regional Clinical Hospital No. 1 (Orenburg, Russia). Semen samples from 72 men were collected during the study period from August 2018 to July 2019.

Our research was performed by strict application of criteria following the 2010 World Health Organization (WHO) guidelines. Sperm parameters were determined manually. To exclude errors in the event of abnormal semen quality at the initial examination, a second examination was carried out after 2 weeks. The total volume of the ejaculate, the color, pH, time to liquefaction; total number of spermatozoa per ml of ejaculate, their motility, morphology, agglutination, and aggregation and the presence of leukocytes,
erythrocytes, and spermatogenic cells were determined. Determination of round cells was the first stage of the study. Subsequently, we differentiated round cells into leukocytes or spermatogenic cells.

Seventy-two subjects were examined. From the data obtained and taking into account anamnesis, the subjects were divided into two groups. Conditionally healthy subjects \((n = 30)\), and infertile subjects \((n = 42)\), in whom changes in the spermiogram of at least two parameters were present and whose partner did not become pregnant after unprotected intercourse for 1 year.

Isolation of strains and determination of microorganism species

A total of 118 strains of microorganisms were isolated from semen using bacteriological methods. Within three hours of collection (in accordance with the WHO recommendations), \(17\) semen samples \((0.1 \text{ ml})\) were inoculated in Endo Agar \((\text{BioMedia, Obolensk, Russia})\), Bi.G.Y. Agar \((\text{Nickerson’s medium; HiMedia Laboratories Pvt., Ltd., Dindori, Nashik, India})\), and Schaedler Agar \((\text{HiMedia Laboratories Pvt., Ltd.})\) and cultured at 37°C. Obligate anaerobic bacteria were cultured in a CO\(_2\) incubator \((\text{Binder, Tuttingen, Germany})\) with O\(_2\) from 0.2% to 0.6% \((v/v)\) and CO\(_2\) from 5% to 9% \((v/v)\). The number of microorganisms in 1 ml of semen was used to determine the colony-forming units \((\text{cfu ml}^{-1})\). Organisms isolated at a concentration of \(>1 \times 10^6 \text{ cfu ml}^{-1}\) were considered significant.

To reliably determine the species of microorganisms, isolated strains were identified by 16 small bacterial ribosomal RNA \((\text{sRNAs})\). Total DNA was extracted by Be\(\text{Ko}v\)a. The samples were supplemented with \(400 \mu\text{l}\) of Tris buffer and homogenized using a TissueLyser LT homogenizer \((\text{Genecraft Labs, Meruya Utara, Jakarta Barat})\) with the E lysing matrix \((\text{MP Biomedikals, Solon, OH, USA})\) for 1 min at 50 Hz. The mixture was then supplemented with 50 ml Tris buffer with lysozyme \((50 \text{ mg ml}^{-1})\) and incubated for 60 min at 37°C. Sodium dodecyl sulfate at a final concentration of 1% and proteinase K were added. The mixture was incubated for 60 min at 60°C. After extraction with phenol-chloroform-isooamyl alcohol \((25:24:1 \text{ v/v/v})\) and subsequent extraction with a chloroform-isooamyl alcohol mixture \((24:1 \text{ v/v})\), DNA from the aqueous phase was precipitated with absolute ethanol with 5 mol l\(^{-1}\) ammonium acetate \((1:10 \text{ v/v})\) at −20°C. DNA was dried and dissolved in Ultra-Pure distilled water \((\text{Invitrogen, USA})\).

DNA libraries were prepared according to the Quick-16S NGS Library Prep Kit protocol \((\text{Zymo Research, Irvine, CA, USA})\). The library was sequenced in a \(2 \times 300\)-nucleotide run using the MiSeq reagent kit version 3 and MiSeq desktop sequencer \((\text{Illumina, San Diego, CA, USA})\). Data analysis was performed on the Local Run Manager platform \((\text{Illumina})\) using 16S Metagenomics Analysis Module version 1.0. The quality of the reads was examined by FastQC version 0.11.7. software \((\text{Shasshot Media, San Diego, CA, USA})\). Paired reads were pooled using PEAR version 0.9.10 software and re-evaluated with FastQC version 0.11.7. The Usearch version 10.0.240 software package was used for filtering and further processing of the pooled reads. Data were filtered with the following parameters: minimum length \((\text{fastq_minlen}) – 420\) base pairs \((\text{bp})\); expected error \((\text{fastq_maxee}) – \text{no more than 1.0}\). Chimeric sequences were removed using the UCHIME2 algorithm. The taxonomy of operational taxonomic units \((\text{OTUs})\) was determined with the NCBI Nucleotide BLAST tool using the RefSeq Targeted Loci database \((https://blast.ncbi.nlm.nih.gov)\).

The method was performed using recombinant cytokines and cell-free supernatants of the studied microorganisms.
The species of microorganisms isolated from the semen of healthy and infertile subjects were different. A total of 118 strains of microorganisms were identified from 72 semen samples, which were divided as follows.

The most abundant bacteria in the healthy subjects was *Staphylococcus* (Table 2), and the least common types were *Corynebacterium, Neisseria, Veillonella*, and *Enterococcus*. The most abundant bacteria in the infertile subjects was also *Staphylococcus*. The least common bacteria in this group of subjects were *Corynebacterium, Streptococcus, Escherichia, Actinomyces, Bacillus, Klebsiella, Pseudomonas, Georgenia, and Propionibacterium* (all *P* < 0.05).

All cell-free supernatants of microorganisms from infertile subjects reduced the level of IL-1, but only some strains reduced the levels of IL-10, TNF-α, IFN-γ, and IL-17 (Table 3). Cell-free supernatants of microorganisms from healthy subjects also decreased the levels of IL-10, IL-1, and IL-17.

Cell-free supernatants of *Staphylococcus* from healthy subjects were more able to reduce IL-10 and IL-17 than those from infertile men (Table 3 and 4). In particular, *Corynebacterium* from healthy subjects reduced IL-10, IL-1 IFN-γ, TNF-α, and IL-17. Cell-free supernatants of *Enterococcus* of infertile men reduced IL-1 levels and *Corynebacterium* reduced TNF-α levels to a greater extent than those from healthy subjects.

We also evaluated the ability of microorganisms isolated from the semen of conditionally healthy individuals and men with infertility to form biofilms (Table 5). *Staphylococcus* and *Enterococcus* species increased the level of biofilm formation in comparison with healthy people. In contrast, *Corynebacterium* reduced biofilm levels in infertile subjects compared to healthy subjects.

### Table 1: Comparison of seminal characteristics of ejaculates between healthy subjects and infertile subjects

| Parameter                  | Healthy subjects (n=30) | Infertile subjects (n=42) |
|----------------------------|-------------------------|---------------------------|
| Semen volume (ml)          | 3.2±1.3                 | 3.4±1.4                   |
| Sperm concentration (x10⁶ ml⁻¹) | 41.4±10.8              | 38.2±18.6                |
| Sperm motility category A + B (%) | 64.1±19.1              | 28.4±12.7                |
| Kruger morphology (%)      | 8.1±2.4                 | 8±0.9                    |
| Leukocyte concentration (x10⁶ ml⁻¹) | 1.8±1.0                | 2.1±0.6                  |
| Spermageneric cells (%)    | 2.4±1.2                 | 3.6±1.3                  |

Significant difference in seminal characteristics of ejaculates in infertile subjects compared to healthy subjects (means±s.d.). s.d.: standard deviation

### Table 2: Species of microorganisms isolated from semen of healthy subjects and infertile subjects

| Stains (healthy subjects/ infertile subjects) | Species composition (%), mean±s.d. | Microorganism (Log cfu per ml semen), mean±s.d. |
|----------------------------------------------|-----------------------------------|-----------------------------------------------|
|                                              | Healthy subjects (n=30) | Infertile subjects (n=42) |                   | Healthy subjects (n=30) | Infertile subjects (n=42) |
| *Staphylococcus* (18/40)                     | 41.8±1.2                  | 53.3±1.8                  | 3.6±0.1            | 5.6±0.1                  |
| *Corynebacterium* (6/11)                     | 14.0±1.0                  | 15.0±1.3                  | 5.0±1.0            | 3.0±0.15                 |
| *Enterococcus* (6/10)                        | 14.0±1.3                  | 13.3±0.9                  | 3.0±0.2            | 5.6±0.16                 |
| *Neisseria* (8/0)                            | 18.6±0.2                  | Not detected              | 8±0.9             | Not detected             |
| *Veillonella* (5/0)                          | 11.6±0.8                  | Not detected              | 5.0±0.2            | Not detected             |
| *Streptococcus* (0/3)                        | Not detected              | 4.0±0.1                   | Not detected       | 3±0.33                   |
| *Escherichia* (0/3)                          | Not detected              | 4.0±0.1                   | Not detected       | 5±0.5                    |
| *Actinomyces* (0/1)                          | Not detected              | 1.3±0.1                   | Not detected       | 3±0.0                    |
| *Bacillus* (0/1)                             | Not detected              | 1.3±0.3                   | Not detected       | 3±0.0                    |
| *Klebsiella* (0/2)                           | Not detected              | 2.6±0.2                   | Not detected       | 3.5±0.5                  |
| *Pseudomonas* (0/2)                          | Not detected              | 2.6±0.2                   | Not detected       | 3.5±0.5                  |
| *Georgenia* (0/1)                            | Not detected              | 1.3±0.1                   | Not detected       | 3±0.0                    |
| *Propionibacterium* (0/1)                    | Not detected              | 1.3±0.1                   | Not detected       | 3±0.0                    |

s.d.: standard deviation; cfu: colony-forming unit

### DISCUSSION

Since semen contains various microorganisms, it can serve as a medium for the development of certain diseases. The presence of bacteria in seminal fluid may represent a potentially curable cause of male infertility. It has been found that a statistically significant negative effect on the reproductive potential of spermatozoa is caused by *Escherichia coli*, *Ureaplasma urealyticum*, and *Staphylococcus aureus*. Therefore, it is important to understand the species composition of semen bacteria to better understand the etiology and pathogenesis of infections and their relationship with infertility.

In our study of the seminal bacterial communities of infertile and healthy subjects, it was shown that the species of microbiota were more extensive in most semen samples from infertile men than those from healthy semen donors. In addition, the species structure in infertile patients consisted of *Streptococcus, Escherichia, Actinomyces, Bacillus, Pseudomonas, Georgenia, and Propionibacterium*. We showed that *Staphylococcus* was more common in the seminal fluid of infertile men, confirming the data of Lbadin and Ibeh, who identified an association of *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and other pathogens with bacteriospermia and poor general sperm motility and morphology. When studying bacteriospermia, other authors have also often isolated *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Other authors, when detecting 16s ribosomal RNA genes, identified four of the more common bacterial species: *Enterococcus faecalis, Escherichia coli*, and *Staphylococcus aureus*. Results combining next-generation sequencing technology and the clinical study of sperm quality have shown that the most numerous bacteria are *Lactobacillus, Pseudomonas, Prevotella, Gardnerella, Rhodanobacter, Streptococcus, Finegoldia*, and *Haemophilus*. The most common genus in healthy subjects in that study was *Corynebacterium*, which is to some extent consistent with our data. In particular, in infertile patients of our study, three main groups of bacteria were found: *Staphylococcus, Corynebacterium, and Enterococcus*. We noted some differences from other authors owing to a combination of two methods: classical bacteriological methods (isolation of a pure culture of microorganisms from semen) and identification of microorganisms based on their 16s ribosomal RNAs.

In addition, various properties of the semen microbiota have been actively studied. It has been shown that semen probably has a highly.
Semen microbiota and cytokines of men

OV Bukharin et al

Table 3: Concentration of secretory inhibitors of cytokines in semen from infertile subjects

| Cell-free supernatant of strains | IL-10 (pg ml⁻¹) | IL-1 (pg ml⁻¹) | TNF-α (pg ml⁻¹) | IFN-γ (pg ml⁻¹) | IL-17 (pg ml⁻¹) |
|----------------------------------|----------------|----------------|----------------|----------------|----------------|
| Staphylococcus (n=40)            | 29.1±4.8       | 243.5±36.4     | 10.37±2.8      | 77.8±11.0      | 170.8±10.3     |
| Corynebacterium (n=11)           | 188.6±32.9     | 247.9±29.1     | 30.43±3.3      | 445.1±51.3     | 351.8±20.5     |
| Enterococcus (n=10)              | 30.5±9.2       | 219.0±40.8     | 9.81±2.0       | 72.7±6.1       | 112.7±3.4      |
| Streptococcus (n=3)              | 20.9±3.4       | 293.8±41.2     | 9.67±0.28      | 72.4±1.6       | 143.5±2.2     |
| Escherichia (n=3)                | 26.2±2.2       | 234.2±20.6     | 9.51±0.3       | 77.6±2.4       | 168.0±6.9     |
| Actinomyces (n=1)                | 30.8±0.0       | 238.4±0.0      | 10.2±0.0       | 92.9±0.0       | 160.7±0.0     |
| Bacillus (n=1)                   | 56.1±1.0       | 115.2±0.0      | 9.53±0.0       | 44.8±0.0       | 65.8±0.0      |
| Klebsiella (n=2)                 | 43.9±5.0       | 164.9±4.4      | 12.23±0.4      | 70.7±1.2       | 126.7±1.0     |
| Pseudomonas (n=2)                | 44.9±2.8       | 96.5±4.3       | 13.1±1.6       | 44.4±2.3       | 99.6±2.6      |
| Georgenia (n=1)                  | 25.6±0.0       | 258.3±0.0      | 9.87±0.0       | 241.9±0.0      | 178.2±0.0     |
| Propionibacterium (n=1)          | 21.8±0.0       | 168.9±0.0      | 14.32±0.0      | 72.6±0.0       | 153.5±0.0     |

1Significant difference in the concentration of secretory inhibitors of cytokines in infertile subjects compared to healthy controls (mean±s.d.). Control level of cytokines: IL-10 (56.1 pg ml⁻¹), IL-1 (316.2 pg ml⁻¹), TNF-α (12.1 pg ml⁻¹), IFN-γ (71.9 pg ml⁻¹), and IL-17 (210.0 pg ml⁻¹). IL-10: interleukin-10; IL-1: interleukin-1; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; IL-17: interleukin-17; s.d.: standard deviation

Table 4: Concentration of secretory inhibitors of cytokines in semen from healthy subjects

| Cell-free supernatant of strains | IL-10 (pg ml⁻¹) | IL-1 (pg ml⁻¹) | TNF-α (pg ml⁻¹) | IFN-γ (pg ml⁻¹) | IL-17 (pg ml⁻¹) |
|----------------------------------|----------------|----------------|----------------|----------------|----------------|
| Staphylococcus (n=18)            | 18.3±2.6       | 255.8±21.6     | 10.5±2.6       | 73.3±18.6      | 18.3±4.6      |
| Corynebacterium (n=6)            | 35.7±4.9       | 239.0±19.5     | 11.7±1.5       | 68.2±10.5      | 35.7±11.8     |
| Enterococcus (n=6)               | 26.9±3.8       | 255.1±17.1     | 9.2±2.0        | 72.5±3.7       | 26.9±4.2      |
| Neisseria (n=8)                  | 17.2±4.2       | 240.8±20.9     | 12.2±3.3       | 68.7±9.3       | 17.2±9.2      |
| Veillonella (n=5)                | 107.6±30.8     | 255.8±18.1     | 21.8±4.1       | 93.6±17.2      | 107.6±30.1    |

1Significant difference in the concentration of secretory inhibitors of cytokines in infertile subjects compared to healthy controls (mean±s.d.). Control level of cytokines: IL-10 (56.1 pg ml⁻¹), IL-1 (316.2 pg ml⁻¹), TNF-α (12.1 pg ml⁻¹), IFN-γ (71.9 pg ml⁻¹), and IL-17 (210.0 pg ml⁻¹). IL-10: interleukin-10; IL-1: interleukin-1; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; IL-17: interleukin-17; s.d.: standard deviation

Table 5: The biofilm-forming capacity of isolated bacterial strains from semen of healthy and infertile men

| Strains (healthy subjects/infertile subjects) | Biofilms produced by microorganisms isolated from healthy subjects (OD₆₃₀) | Biofilms produced by microorganisms isolated from infertile subjects (OD₆₃₀) |
|-----------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Staphylococcus (18/40)                        | 0.46±0.18                                                                      | 0.94±0.14                                                                      |
| Corynebacterium (6/11)                        | 0.45±0.12                                                                      | 0.21±0.09                                                                      |
| Enterococcus (6/10)                          | 0.77±0.12                                                                      | 1.05±0.21                                                                      |
| Neisseria (8/0)                               | 0.46±0.10                                                                      | Not detected                                                                    |
| Veillonella (5/0)                             | 0.30±0.16                                                                      | Not detected                                                                    |
| Streptococcus (0/3)                           | Not detected                                                                    | 0.82±0.21                                                                      |
| Escherichia (0/0)                             | Not detected                                                                    | 0.80±0.13                                                                      |
| Actinomyces (0/1)                             | Not detected                                                                    | 0.14±0.00                                                                      |
| Bacillus (0/1)                                | Not detected                                                                    | 0.82±0.00                                                                      |
| Klebsiella (0/2)                              | Not detected                                                                    | 0.90±0.06                                                                      |
| Pseudomonas (0/0)                             | Not detected                                                                    | 1.29±0.20                                                                      |
| Georgenia (0/1)                               | Not detected                                                                    | 0.14±0.00                                                                      |
| Propionibacterium (0/1)                       | Not detected                                                                    | 0.87±0.00                                                                      |

1Significant difference in bacterial biofilms between healthy and infertile subjects (mean±s.d.). OD₆₃₀: optical density at 630 nm; s.d.: standard deviation

Effective defense system involving the production of microbiocidal agents, likely comprising peptides or proteins.5,34

The secretion of the secretory inhibitor of platelet microbicidal protein (SIPMP) by urethral isolates has also been shown to be correlated with the diagnosis of prostatitis. Moreover, the ability of bacteria to inactivate lysozyme is associated with the development of infertility.35,37

Staphylococcus, Corynebacterium, and Enterococcus isolated from infertile subjects have higher levels of biofilm formation than the same bacteria isolated from healthy men. Other equally important studies related to the study of biofilms of microorganisms in semen have shown that for the fixation of a biotope, bacteria must attach to surfaces of the host.38,39 Thus, it is necessary to determine the level of biofilm-producing strains isolated from the seminal fluid of infertile subjects.

The presence of various microorganisms in the seminal plasma of healthy and fertile males should be considered from the perspective of their interaction with local antimicrobial defense factors of the host.

Inflammation as a response to an incoming foreign agent involves a complex interplay of signaling molecules known as cytokines and their receptors expressed by host tissues and immune cells. Cytokine networks are the main controlling elements in inflammation and immune reactions that occur in infections. Consequently, these cytokine mediators are often targets of invading microorganisms, disrupting the immune response.

Complex interactions between proinflammatory and anti-inflammatory mediators allow the proper balance to be maintained and prevent the development of pathological, tissue-destructive processes.40 Therefore, the interaction between bacteria and cytokine networks is an important theme in microbial pathogenicity. Disturbance of this communication network has the potential to profoundly affect the pathological outcome of infectious diseases. One mode of cytokine signaling disruption can occur through proteolytic modification of these molecules and their receptors on cell surfaces.

It has been reported that lysine-specific gingipain Porphyromonas gingivalis in the coculture system degrades and inactivates TNF-α, IL-1β, IL-1 receptor antagonist, IL-6, IL-8, IFN-γ, and IL-17A.41,42

Group A Streptococcus (GAS) protease SpyCEP cleaves IL-8. By inactivating the multifunctional host defense peptide IL-8, the SpyCEP protease impairs neutrophil clearance mechanisms, contributing to the pathogenesis of invasive streptococcal infection.43

Pseudomonas aeruginosa secretes several proteases that are considered to be important virulence factors. Two key proinflammatory cytokines, IL-6 and IL-8, are substrates for pseudolysin (elastase) and aeruginolysin (alkaline protease).44 This phenomenon could help Pseudomonas aeruginosa establish an infection, since it blocks the
recruitment of leukocytes. Pseudomonas aeruginosa isolated from cystic fibrosis subjects secretes the protease AprA in a LasR-dependent manner, which is able to directly degrade epithelial-derived IFNA, inhibiting IFN signaling.46

Cytokines are physiological seminal components, and their abnormal levels, reported in different pathological conditions, disrupt semen function.47 Many so-called proinflammatory and immunoregulatory cytokines are produced by testicular somatic and spermatogenic cells, both under normal conditions and in response to inflammatory stimuli.48 Seminal cytokines are also secreted by immunocompetent cells in various pathogenic conditions, including leukocystospermia.49

In our study, microorganisms, such as Staphylococcus and Corynebacterium, were able to inactivate IL-10. The genus Corynebacterium in infertile men, in comparison with conventionally healthy people, also reduced the level of the secretory inhibitor of cytokines in relation to TNF-α and the Enterococcus reduced the level of the secretory inhibitor of cytokines IL-1.

This study raises further questions. Can an increase in microbial cytokines affect the local cytokine balance? Thus, further research is required to determine whether a causal relationship exists between microorganism cytokines (IL-10, IL-1, TNF-α, IFN-γ, and IL-17) and male infertility. The answer to this question lies in continuing research in clinical trials.

AUTHOR CONTRIBUTIONS
OV Bukharin conceived of the study and participated in its design and coordination. NBP participated in the project coordination, generalization of experimental data, and literature search. EVI identified the isolated microorganisms by genetic methods and bioinformatics processing and helped draft the manuscript. INC studied the secretory inhibitor of cytokines of microorganisms isolated from semen. AVB performed the bacteriological isolation of microorganisms from semen. TAB isolated microorganisms to form biofilms. MDK collected biomaterials and coordinated the clinical coordination. NBP participated in the project coordination, male infertility. The answer to this question lies in continuing research.
Semen microbiota and cytokines of men
OV Bukharin et al

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