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**Vladimer Papava**  
TSMU Department of Urology,  
assistant-professor. MD, PhD,  
Tbilisi, Georgia

**Tamar Didbaridze**  
Microbiologist. TSMU the First  
University, Clinic.MD,  
PhD,Tbilisi, Georgia

**David Kochiashvili**  
Head of Department of  
Urology, TSMU, Professor.  
MD, PhD, Tbilisi, Georgia

**Correspondence:**  
**Vladimer Papava**  
TSMU, Department of  
Urology, assistant-professor.  
MD, PhD, Tbilisi, Georgia

## Influence of facultative bacteria on semen parameters

Vladimer Papava, Tamar Didbaridze, David Kochiashvili

### Abstract

Infections of the male genitourinary tract (GUT) account for up to 15% of cases of male infertility. Sperm bacterial contamination is quite frequent and could contribute to the deterioration of the sperm quality of infertile men. Many studies have examined the impact of genital tract infections and bacterial semen contamination in male fertility. Microorganisms can affect the male reproductive function directly, causing the agglutination of motile sperm. The most frequently isolated microorganism in male patients with genital tract infections or semen contamination are non-specific (facultative) bacteria, mainly Enterobacteriaceae e.g. *Escherichia coli* *Staphylococcus* spp, *Streptococcus* spp, *Klebsiella* spp, and yeast-like cells. The aim of this study was to investigate the semen quality in the presence of different facultative bacterial species. Semen samples were processed for bacteriological analysis and examined to evaluate sperm concentration and motility and to verify the prevalence of semen bacterial contamination and whether the contamination could decrease sperm quality. We studied 58 men (64, 4%) who had bacteriologically positive culture in seminal fluid and no other risk factor for infertility. Sperm motility and count were reduced in all infertile men with positive bacteriological results and were significantly improved after adequate therapy.

**Keywords:** non-specific bacteria, semen quality, GUT infections

### Introduction

The negative influence of bacteria on sperm motility is well known. Microbial genital tract infections could be specific (*Chlamydia trachomatis*, gonorrhoea caused by *Neisseria gonorrhoea*, ureaplasmosis caused by *Ureaplasma urealyticum* and trichomoniasis caused by *Trichomonas vaginalis*) and non-specific (facultative) aetiology (mainly by: *Enterobacteriaceae* e.g. *E. coli*), *Staphylococci*, *Streptococci*, *Klebsiella* spp., and yeast-like cells (a fungus) (1, 2). Infections of the male genitourinary tract account for up to 15% of cases of male infertility (3). Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process (4, 5), causing qualitative and quantitative sperm alterations. Recent studies have shown that the simple presence of bacteria in semen samples may compromise the sperm quality. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse. Mehta et al. (6) reported that aerobic cocci are present in about 62% of semen samples of male partners in infertile couples. *Enterococcus faecalis* was isolated from 53% of patients, micrococci from 20% and alpha-haemolytic streptococci from 16% of the infected samples. Increased prevalence of genital tract infections caused by *E. faecalis* is associated with compromised semen quality in terms of sperm concentration and morphology. The presence of micrococci and alpha-haemolytic streptococci does not appear to exert any detrimental effect on sperm quality. (7, 8). Bacterial flagella and pili could be an important determinant of pathogenicity. Bacteria isolated from the site of infection are more likely to be piliated, while when they are simple bystanders they are not; this is the case of bacteria colonizing the urethral tract of human beings (9, 10). Although no significant depressor effect of enterococci on sperm motility was observed, some researchers described, in an *in vitro* study, a negative influence on membrane integrity of human sperm head, neck

and mid-piece (11), probably mediated by hemolysin, a well-known virulence factor of enterococci. The presence of leukocytes in the semen often complicates the interpretation of results of sperm analyses and alterations of sperm parameters (12, 13).

Genital ureaplasmas and mycoplasmas may colonize male urethra and contaminate the semen during ejaculation. However, these microorganisms and particularly *Ureaplasma urealyticum* are potentially pathogenic species playing an etiologic role in both genital infections and male infertility (14). *U. urealyticum*, one of the most frequent causes of the male infertility (15), due to its ability to reduce semen quality and the fertilizing potential of sperm, negatively influences the sperm motility, density and morphology and reduces the oxidoreductive potential of the ejaculate, which makes sperm more vulnerable to peroxidative damage. The mechanisms of sperm damage caused by bacteria passes through the expression of the adhesive properties of the flagella and pili to mannose receptors (16). The fact that receptors to mannose have been demonstrated also at the surface of human spermatozoa (17) suggests that flagella and pili could play a considerable causative role in sperm damage. The gram positive organisms are concerned, the production of pili is not a characteristic present in all clones of the same species, pili could possibly constitute a putative determinant of pathogenicity in gram positive cocci, too (18, 19).

The cause-effect relationship between bacterial infections and semen contamination and male infertility is still being debated. To complicate the problem even more, the presence of bacteria in semen samples of infertile men has a similar prevalence to that observed in fertile males (20). The clinical significance of bacteria in semen is still unclear (21).

The aim of this study was to investigate the semen quality in the presence of different facultative bacterial species. Semen samples were processed for bacteriological analysis and examined to evaluate sperm concentration and motility.

### Material and Methods

We retrospectively have studied the medical records of the 90 patients who visited TSMU the First University Clinic Department of Urology from 2017 January - until June 2017 with diagnoses of infertility. Standard analysis were performed: 1 Spermogram by using Sperm Quality Analyzer (SQA IIC-P). Semen was collected after 3-4 days of sexual abstinence in aseptic condition in clean dry, sterile container at least twice. 2. Ultrasound of scrotum (to excepted varicocele). 3. Analysis following hormones: Testosterone, FSH, LH, Prolactin (PRL). Analysis of HbsAg, Anti - HCV, Anti TP, Anti -HIV in all patients were negative. We also studied Analysis bacteriology of seminal fluid. Among this patients we studied only 58 who had bacteriologically positive culture in seminal fluid and no other risk factor for infertility. Patients were asked to urinate and wash the hands, penis and scrotum before ejaculation to avoid possible contamination from the urine or external genitalia. The sample was taken to the laboratory for further analysis without any delay. Samples were seeded using a calibrated loop on agar plates, which were incubated overnight at 37°C in normal air with 5% CO<sub>2</sub>. The microorganisms were identified by gram stain, oxidase, catalase and other biochemical tests using Bio-

Mérieux products (API Staph, API 20E, API-20 Strep, Api Caux, Bio-Mérieux). Spermicocultures were considered positive when the number of colonies was  $\geq 10^4$  CFU ml<sup>-1</sup> in case of gram positive cocci and  $\geq 10^5$  CFU ml<sup>-1</sup> in case of gram negative rods. Semen volume, sperm concentration, progressive motility and leukocyte concentration were evaluated according to WHO guidelines.

### Results

Spermicoculture in the 58 patients yielded *Staphylococcus aureus* in 19(32,8%, 15 men were oligoasthenospermic, 4-azoospermic), *Enterococcus faecalis* in 13 samples (22,4%, 10 men were oligoasthenospermic, 3-asthenospermic) *Staphylococcus epidermidis* in 10 samples (17,2%, 10 men were oligoasthenospermic), *Streptococcus anginosus* in 6 specimens (10.3%, 6 men were oligoasthenospermic), *Escherichia coli* 6 in samples (10,4%, 5 were asthenospermic, 1-oligospermic), *Enterobacter cloacae* in 4 samples (6,9%, 4 men were oligospermic).

### Conclusion

Sperm bacterial (facultative) contamination according our study were in 64, 4 % (58 patients) infertile men. They had no other risk factor for infertility. This bacteria contributed to the deterioration of the sperm quality. Gram-positive cocci were prevailed. In our study, sperm motility and count were reduced in all infertile men with positive bacteriological results. *All patient underwent 21 day antibiotic therapy depends on local susceptibility test and vitamins and minerals were added to the treatment for 3 month. After appropriate therapy sperm quality (morphology, quantity, also spermagglutination) were significantly improved.*

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