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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

Valeri Khvakhajelidze
TSMU Department of Urology, Urologist. Tbilisi, Georgia

Leli Shanidze
Manager of the Georgian-Austrian Medical Centre, Lab Doctor, MD Tbilisi, Georgia

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

Isolation of Staphylococcus aureus in seminal fluid and its impact on semen quality/quantity

Vladimer Papava,Tamar Didbaridze,Valeri Khvakhajelidze, Leli Shanidze

Abstract
The influence of Gram-positive uropathogenic bacteria on sperm morphology and function has been poorly investigated until now.The effects of bacterial presence on sperm quality and fertility are controversial. *S. aureus* is one of the dominant bacteria isolated from the semen samples of males complaining of infertility. Bacteria are capable of agglutinating and immobilizing spermatozoa. *S. aureus* reduced sperm motility. Although *S. aureus* has been reported to cause immobilization of spermatozoa, however, its role in infertility has yet to be elucidated.
We retrospectively have studied the medical records of the 28 patients who visited TSMU The First University Clinic Department of Urology from 2017 January - until May 2017 with complaining of infertility and who had bacteriologicaly Staphylococcus aureus in seminal fluid, no other urophatogens(Ureaplasma, Chlamidia, Mycoplasma) and risk factors for infertility were found and determined the influence of Staphylococcus aureus on semen quality and quantity.

Keywords: Staphylococcus aureus, Semen quality, Infertility

Introduction
S. aureus is one of the dominant bacteria isolated from the semen samples of males complaining of infertility. It has the ability to cause a variety of infections in numerous ecological niches within the host. It colonizes the nares, axillae, vagina, pharynx, or damaged skin surfaces and causes a variety of suppurative (pus-forming) infections and toxinoses in humans. Besides this, S. aureus is arguably the dominant organism implicated in primary infertility, among males and females (1, 2, and 3). S. aureus has been observed as causative organism accounting for 68.2% of seminal fluid infections (4, 5). This is consistent with that reported by Okon et al., where S. aureus was isolated from 62.5% of the seminal fluids (6, 7, and 8). Infection with different microorganisms, such as chlamydia, mycoplasma, and certain uropathogenic bacteria, may lead to various clinical manifestations of human reproductive function.Some authors have suggested that direct interaction between bacteria and spermatozoa facilitates immobilization of spermatozoa (9,10,), while others have reported evidence for soluble spermicidal factor produced and secreted by bacteria in the extracellular medium (11).
Controversies exist in literature regarding the role of bacterial infection in infertility. Barring the role of a few bacteria such as Chlamydia whose impact on fertility has been well established, the role of other bacteria in infertility is debatable (12, 13). Though, many microorganisms have been isolated from seminal fluid samples of infertile patients, but it has not been clearly established as to whether any of the organisms isolated actually causes infertility. Momoh et al. (14,15) reported that *Staphylococcus aureus* is one of the dominant microorganism with a prevalence rate of 38.7% from high vaginal swab and endocervical swabs, respectively and a prevalence of 75% among the bacterial strains from semen cultures of infertile couples(16, 17, and 18).
Infections of the male genitourinary tract account for up to 15% of cases of male infertility. Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process,causing

qualitative and quantitative sperm alterations(19,20). 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 (21, 22) Various studies have shown *Staphylococcus aureus* to be one of the most prevalent organism in male and female genital tract but most practitioners dismiss it as mere contamination which is assumed to be of no significance (23, 24). However, it is now suggested that the presence of this organism should not be ignored, as incubation of spermatozoa with *S. aureus* results in reduced sperm motility. Although *S. aureus* has been reported to cause immobilization of spermatozoa, however, its role in infertility has yet to be elucidated (25, 26).

Aim of our study was to determine the influence of Staphylococcus aureus on semen quality and quantity.

Materials and Methods

We retrospectively have studied the medical records of the 28 patients who visited TSMU the First University Clinic Department of Urology from 2017 January - until May 2017 with diagnoses of infertility.Standard analysis were performed:

1. Spermogram by using Sperm Quality Analyzer (SQA IICP) at least twice.
2. Ultrasound of scrotum (to excepted varicocele).
3. Analysis following hormones: Testosterone, FSH, LH, Prolactin (PRL).
4. Safe blood analysis of HBsAg, Anti – HCV, Anti TP, Anti -HIV
5. Bacteriology of Seminal fluid

Safe blood tests in all pacients were negative. They had hormonal balance in normal level. We also We focused on 28 patients who had bacteriologically Staphylococcus aureus in seminal fluid and no other urogenital phatogens such as Chlamydia, Ureaplasma and Mycoplasma. Spermiogram, semen culture and oxidative stress test were performed. Semen was collected after 3-4 days of sexual abstinence in aseptic condition in clean dry, sterile container. 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_2. The microorganisms were identified by gram stain, oxidase, catalase and other biochemical tests using Bio-Mérieux products (Bio-Mérieux). Spermiocultures were considered positive when the number of colonies was $\geq 10^4$ CFU ml^{-1} in case of gram positive cocci. Sensitivity of microorganisms to antibiotics was defined with Kirby-Bauer disc-diffusion method using standard discs (EUCAST guidelines 2017). Antibiotic susceptibility test was done on following antibiotics: amoxicillin+clavulanic acid, ampicillin+sulbactam, amikacine,cefazoline,cefoxitine,norfloxacine, ciprofloxacine, levofloxacine, moxifloxacine, fosfomycine, doxycycline, azithromycine, nitrofurantoin, thrimethoprim-sulfamethoxazole.

Semen quality was evaluated on Sperm Quality Analyzer(SQA IIC-P) by measuring semen volume(ejaculate in ml), ph, liquefaction, viscosity, motility,morphology, and concentracion(WHO standard(a + b = >50%) and control RPM (a) = 55.3%.),sperm concentration and polymorphonuclear leukocytes and other white blood cells. Samples were examined after liquefaction for 30 min at 37°C. Volume, pH, concentration and motility were evaluated according to World Health Organization guidelines WHO (19).

Parameters	Norm by WHO standards
Volume	Non less than 2 ml
Color	White, grayish, yellowish
Viscosity/consistence	Dropping, drop up 2 cm
Liquefaction	10 – 40 min
pH level	7.2 - 8.0
Concentration *Quantity of spermatozoa in 1ml*	More than 20 M
Count *Quantity of spermatozoa in ejaculate*	More than 40 M
Motility	Progressive motile – more than 32% Total count pf motile spermatozoa – more than 40%
Round cell concentration *Cells of spermatogenesis: leucocytes, macrophages, epithelial cells, immature generative cells*	Up to 2%
Agglutination *Adhesion of spermatozoa*	None
Morphology *Build of spermatozoa*	Quantity of normal spermatozoa is more than 4%

Leukocytes were identified by peroxidase stain; WHO (19) considered normal a leukocytes concentration $\leq 10^6$ cells/ ml. Peroxidase-positive leukocytes include polymorphonuclear leukocytes (50%~60%) and macrophages (20%~30%). A large proportion of these peroxidase-positive leukocytes originate from the prostate and seminal vesicles. When these major sources of reactive oxygen species (ROS) are activated by various intracellular or extracellular stimuli, such as infection or inflammation, they can discharge up to 100 times more ROS than normal and increase the NADPH production via the hexose monophosphate shunt. An increase in proinflammatory cytokines, such as interleukin (IL)-8, and a decrease in the antioxidant superoxide dismutase (SOD) can result in a respiratory burst, production of high levels of ROS, and ultimately, OS. Oxidative Sstress will cause sperm damage if seminal leukocyte concentrations are abnormally high as is the case in leukocytospermia, which the World Health

Organization defines as the presence of more than one million peroxidase-positive cells per milliliter of semen.

Results

On semen quality/quantity, we report 52 % oligospermia, 15% azoospemia and 33% of normospermia. In oligospermic and azoospermic who bacteriologicaly had Staphylococcus aureus 10^8/ml oxidative stress were L4 (high). In normospermic patients oxidative stress were L3 (medium).

Antimicrobial treatment was administrated for 3-4 weeks depends on local susceptibility tests. In order to esteem success of treatment for infertility we made control spermogram, bacteriology and oxidative stress test. Semen analysis show significant improvement of sperm quality, morphology and quantity.

Oxidative stress were reduced in patients who had L4 to L1 and L2, and from L3 to L1.

Conclusions

Generally, the semen quality (vol., rapid progressive motility, sperm concentration and immotility) for all patients who had bacteriologicaly Staphylococcus aureus in semen were significantly low. Bacterial presence in semen reduced mean sperm concentration and viability, thereby contributed to oligospermia, azoospermia and also influence on oxidative stress making it increase to L4 wich elevates sperm chromatin/ DNA damage and interact with lipids, proteins.Adequate antimicrobial treatment is effective to improve sperm quality/quantity.

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