

CASE REPORT

Genitourinary Bacterial Infection: a Cause of Infertility in Men? A Cases Series and Short Review of Literature

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Introduction: The role of bacterial infections on the onset and the development of male infertility is still highly controversial, as the clinical cases have different outcomes and the practitioners have no guidelines that will help them select the cases that could better benefit from antibiotic treatment. **Case presentation:** Four case reports are presented in order to emphasize the possible clinical implications of genitourinary bacterial infections on male infertility. The first patient had two bacterial strains isolated from the semen culture, *Escherichia coli*, and then *Enterococcus faecalis*. The antibiotic treatment was not effective. The second patient had a semen culture positive with *Enterococcus faecalis*. The treatment was successful: the bacteria were eradicated and the patient was able to conceive a baby. *Enterococcus faecalis* was also identified in the third and the fourth case. These patients were able to conceive, despite the different clinical management strategies of the cases. **Conclusion:** Bacterial prostatitis might play a role in male infertility, but the clinical cases are still highly unpredictable. Every case presents a different viewpoint and raises awareness of the complexity of the problem.

Keywords: *Enterococcus faecalis*, spermatozoa, azoospermia, male infertility, sperm count

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Introduction

At least 30 million men are infertile around the globe, making male infertility a subject of real concern [1]. The complex etiology of the problem, combined with the public stigma that might come from acknowledging and addressing this problem, makes this a sensitive issue. A meta-analysis targeting men from Europe, North America, and Australia reported a 50-60% decline in sperm quality during 1973-2011, with vast implications on men's health [2].

Globally, 15% of couples are affected by infertility, 20-30% of which are due solely to male infertility, while 50% are due to causes that involve both partners [1, 3, 4]. The highest infertility rates were reported in Africa and Central/Eastern Europe [1].

According to De Kretser and Baker, the male infertility etiological factors are classified into 3 categories: pre-testicular (endocrine disorders, coital disorders, or ejaculatory failure), testicular (infective pathologies, genetic or congenital disorders), and post-testicular (genital tracts obstructions or accessory gland infections). Even though this classification was published in 1999, it is still widely used [5].

Bacterial infections might play a role in male infertility. A study published in 2009 by Moretti et al, showed that the most frequently isolated bacteria that might affect the

quality of spermatozoa are *Enterococcus faecalis* (32.1%), *Escherichia coli* (20.1%), *Streptococcus agalactiae* (13.4%), and *Ureaplasma urealyticum* (11.8%) [6].

Sperm quality is closely linked with infertility, although not exclusively. Urogenital bacterial infections are responsible for 15% of the male infertility cases and some authors consider that the presence of bacteria in semen may affect the quality of spermatozoa [7], but there are still many unanswered questions on this topic.

The main reason for this mini-series of case reports is to present the possible different evolution of genitourinary tract infections and the possible impact that bacterial prostatitis might have on the quality of the semen.

Materials and methods

Prior to harvesting the clinical sample, all four patients followed the World Health Organization 2010 protocol: 3 to 7 days of abstinence, proper hygiene before collecting the specimen and transportation time to the laboratory of less than 30 minutes. After receiving the samples in the laboratory, these were incubated at 37°C for up to one hour, to establish the liquefaction time.

The motility, viability of spermatozoa, the presence of bacteria or leukocytes were evaluated by wet mounts. To count the number of spermatozoa, we used solutions containing water and formaldehyde in a ratio of 9:1.

The spermatozoa morphology was evaluated by Giemsa staining.

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The bacterial cultures and antibiotic susceptibility testing were performed following the current protocols that are used in the laboratory. Shortly after their arrival, the samples were inoculated on specific culture media, incubated, and the colonies bacterial strains were identified based on their biochemical characters. The antibiotic susceptibility was performed following EUCAST standard.

The study was approved by the Medical College of Romania (decision no.3374/28.07.2020).

Presentation of case series

Case 1

A 28-years-old patient presented to the medical laboratory in March 2018 for a semen analysis. The patient and his wife confirmed that they were trying without success to have a baby for longer than 6 months. The wife's laboratory tests and her gynecological consult were in the normal range.

The Family Practitioner recommended the husband to perform a sperm count. The patient denied any other illnesses. Before the sperm count, the patient complied with all the pre-analytical requirements established by the laboratory. The sample volume was 3.1 ml, the viscosity and the appearance were both in the normal range, the liquefaction occurred at 25 minutes and the pH was 8.5.

The microscopical examination showed no spermatozoa but a field full of bacteria, polymorphonuclear (PMN) leukocytes, and few red blood cells. Semen culture showed a pure and rich culture of *E. coli*, and the isolated strain was susceptible to all tested antibiotics.

The patient was reassessed after two months. At that time, he was following the treatment with gentamicin, trimethoprim - sulfamethoxazole and nystatin for 7 days, as prescribed by his urologist.

The semen analysis showed the following: a volume of 2.5 ml, a viscosity and pH similar values as in the first sample, 2-3 viable spermatozoa per microscopic field with grade B motility. Also, the sample contained numerous sperm precursor cells, PMN leukocytes, and bacteria. No bacterial culture was performed, as the patient was already following antibiotic therapy. The prolongation of the treatment for up to 21-30 days was recommended, but not prescribed.

After finishing the antibiotic treatment, the patient followed a complementary treatment with an amino-acids-containing drug that enhances the fertility rate. The patient returned to the laboratory for reevaluation, but no improvement in the quality of the semen was noticed. Only 2-3 viable spermatozoa with grade A and B motility were observed per microscopical field, along with bacteria, PMN leukocytes, and sperm precursor cells. The bacterial culture showed *E. faecalis*, susceptible to all tested antibiotics.

In order to exclude potential sample contamination, a second sample was harvested and processed. In the second

sample, the microscopy revealed 15 spermatozoa/field with grades A and B motility, frequent bacteria, and PMN leukocytes. A pure culture of *E. faecalis* was again identified. The patient's wife had *E. faecalis* in her vaginal secretion. Treatment with 1 gram of ampicillin per day for 30 days was suggested, alongside anti-mycotic prophylaxis with 150 mg of fluconazole every 4 days.

At reevaluation in January 2019, after the patient finished the treatment, the sperm count showed 6-8 spermatozoa per microscope field (motility grade B, C, D, and rarely, grade A). *E. faecalis*, susceptible to all tested antibiotics was again isolated. The difference between this sample and the previous ones consisted of the lack of inflammatory cells.

A standard dosage of hormones, spermatozoa antibodies, and a testicular echography for a possible varicocele was recommended. The FSH and testosterone values were out of range, so the patient was referred to the Endocrinology Department where a treatment was prescribed. The treatment did not improve the patient's fertility status.

In April 2019, the following were observed on the semen analysis: high viscosity, a volume of 4 ml, 4-5 spermatozoa per microscope field with grade B motility, and a high inflammatory response. In the semen culture, a strain of *E. faecalis* with the same susceptibility to antibiotics was identified.

Case 2

A 32-years-old patient presented to the Medical Laboratory for performing a semen analysis and a semen culture. He declared that he and his wife were trying to conceive without success for more than 1 year. On semen count, azoospermia was observed, as well as bacteria and inflammatory cells. The sample volume was low, with a high pH. A pure culture of *E. faecalis* was noted. The patient followed a treatment with levofloxacin 500 mg for 30 days, and diclofenac suppository for 7 days. The patient turned back after he finished the treatment. The sperm count was in the normal range, passing from azoospermia to normozoospermia with over 45 million spermatozoa. No bacterial growth was observed. Following this, the couple managed to conceive a child.

Case 3

A 29-year-old patient presented at the Medical Laboratory for a semen analysis and a semen culture on his own will. The reason he wanted to take the test was the low volume of ejaculate during intercourse. The urologist discovered a severe prostate inflammation and calcification of some prostate zones. The sperm analysis showed a low sample volume (1.8 ml), with high viscosity and normal pH. Microscopy showed 14.6 million/ml highly mobile spermatozoa with grade A and B motility. Inflammatory cells and bacteria were present. The semen culture revealed *E. faecalis* in pure, rich culture. The patient followed a treatment with 500 mg levofloxacin for 30 days, but the treatment was unsuccessful. The number of spermatozoa after the

treatment did not increase significantly, but the volume of the probe was 3.2 ml. After 4 months, the patient managed to conceive a child.

Case 4

A 26-year-old patient presented to the Medical Laboratory for a prophylactic semen analysis on his own will. At the first impression, the volume, the pH, the viscosity, and the appearance of the semen were in the normal range. On microscopy, normozoospermia, inflammatory cells, and bacteria were observed. From the semen culture, a pure culture of *E. faecalis* was obtained. The patient refused to follow a treatment because of the lack of symptoms. A short time later, the patient had a child.

Discussion

Even in the 21st century, sperm count and sperm culture is a taboo subject for a lot of young men. Most of the patients perform sperm analysis only when they fail in conceiving, and when all the female infertility factors are excluded. Thirty-seven percent of females might have factors that affect the capability of conceiving a baby [8], but most of the doctors consider female dysfunctions to be the main reason for conceiving failure. Oprea et al. showed that 1 in 10 men have azoospermia and from a total of 109 semen counts only 23% were in the normal range [9].

Most couples get worried after they try a couple of months to conceive a baby, with no success. Women under 35 years old are considered infertile after they try to conceive a baby for longer than one year. For women over 35 years old, on the other hand, infertility is suspected after 6 months [10]. As in the first presented case, the male subject presented at the lab to perform a sperm count only after his wife had a gynecological consult, and no medical issues were discovered.

It is very hard to collect the sample in a sterile way, as the commensal urethral and penile microflora contain a large number of bacteria. According to Bowie et al., the gland is colonized with Gram-positive aerobic bacteria, coagulase-negative staphylococci, or alpha-hemolytic streptococci [11]. Schneider et al. showed that *Staphylococcus aureus*, Enterobacterales (*E. coli*, *Klebsiella* spp.), *Enterococcus* spp. and even *Pseudomonas aeruginosa* are part of the normal penile flora [12]. This makes it difficult to interpret a positive genital bacterial culture, especially in the absence of the symptoms. Improper harvesting techniques induce false-positive results so, before sperm analysis, the patient has to be counseled for a proper specimen collection. More specifically, one day before collecting the sample, the patient should wash the genital area with soap and water. On the testing day, the patient should wash the penis gland only with water, urinate, and then wash his hands before collecting the sperm.

In the first case presented, *E. coli* might have been part of the chronic infection, at least promoting it aside from *Enterococcus faecalis*. After treatment and eradication of *E.*

coli, spermatozoa count increased, but not significantly. Even if the patient followed multiple treatments with different antibiotics, the treatment was ineffective on *E. faecalis*, which was still present in the semen culture. Nevertheless, the low sperm count is less probable to be explained solely by the presence of *E. faecalis*, so hormonal analysis and endocrine examination were further recommended. Indeed, low levels of follicle-stimulating hormone (FSH) and testosterone were found later.

In this case, the urologist first recommended intramuscular gentamicin associated with trimethoprim - sulfamethoxazole orally, based on the antibiotic susceptibility results. According to the urology guidelines, the standard treatment for chronic prostatitis is 500 mg levofloxacin for 4-6 weeks. In the patient's case, according to the EUCAST antibiotic susceptibility testing guidelines, levofloxacin is not standardly tested, as it is recommended to use levofloxacin only in uncomplicated urinary tract infections [13].

After treatment, the inflammatory response decreased, and *E. coli* infection was remitted, but afterward *E. faecalis* appeared, with no inflammatory response for a short time. This could be because *Enterococcus* spp. is a commensal pathogen that may induce chronic prostatitis but in comparison to *E. coli*, it has lower pathogenicity.

In the second case, the presence of *E. faecalis* was most probably the etiological agent, and the state of infection is clear as the sperm count returned at normal values after treatment. The treatment with 500 mg levofloxacin was efficient, although the susceptibility to this antibiotic was not tested *in vitro*. If the treatment with diclofenac suppository had a real benefit for the inflammatory process, remains an unanswered question.

In the third case, although the patient had a low volume of semen, and the sample contained *E. faecalis* and PMN leukocytes, he was able to conceive a baby. One possible explanation is that despite the low number of spermatozoa, they maintained their motility. Whereas the presence of *E. faecalis* can be considered colonization or infection is still open for debate. Can colonization influence the quality of spermatozoa, and if yes, when? Moretti et al. have demonstrated that the presence of bacteria, even without an inflammatory response may affect the semen quality [6].

It must be mentioned that all four patients did not present clinical symptoms. Before beginning the analysis, all four patients completed a questionnaire denying the presence of symptoms like a difficult or a burning sensation during ejaculation, a change in the smell of sperm, or any kind of pain.

The immune response can help the body fight foreign antigens, and in some cases, it can even target self-cells. Some researchers suggest that the presence of leukocytes does not affect sperm quality [15]. A concentration of more than 2×10^6 leukocytes/ml has improved the motility of spermatozoa [16]. Despite this, most authors agree on the indirect negative effect of inflammation on sperm quality [17].

The presence of leukocytes should have not affected the conceiving in three from the four cases presented. Leukospermia can be present in both fertile and infertile men [16]. More than that, the infection with *Enterococcus* spp. is associated with a lack of symptoms.

Bacteria can affect the quality of the semen with the help of their virulence factors. *Enterococcus* spp. are producing hemolysins that damage the cell membrane, at least *in vitro*, but the role that these hemolysins play *in vivo* is still incompletely elucidated [18]. Hemolysins are not the only virulence factors produced by *Enterococcus* spp. Biofilms, for example, are complex tri-dimensional structures that favor the colonization state, by enabling bacteria to attach to other cells [19]. Adhesins like LPxTG (a surface protein) and pili are also key players in the colonization process. Moreover, *Enterococcus* spp. produce aggregation substances, which help them to adhere to the cells [21] and to survive inside inflammatory cells like macrophages and PMN, after phagocytosis [24].

Additionally, microbial surface component that recognize the adhesive matrix (MSCRAMMs) mediate adhesion to the extracellular matrix and contribute to the attachment to the cells [22]. In urinary tract infections, both *E. faecalis* and *E. faecium* have surface proteins like *esp* with an important role in the colonization of the urinary tract [23].

Bacteria can slow down the motility of spermatozoa by attaching to the sperm cell, it can produce toxins like alpha-hemolysins, beta-hemolysins, lipopolysaccharides, or trigger an immune response [17]. By attaching to the spermatozoa plasma membrane, the bacteria can modify its structure and damage the acrosome [25]. Even in procedures like IVF (in vitro fertilization) the contamination with bacteria results in the degradation of the oocyte [26].

Generalized acute or chronic infections have been associated with steroidogenesis and spermatogenesis, producing temporary or permanent infertility. The human body responds to the presence of bacteria rapidly, synthesizing mediators, cytokines, and growth factors and then releasing them in the seminal plasma. In male accessory gland infections, for example, the presence of leukocytes in semen is associated with modified levels of -glucosidase and -glutamyl transferase, and with a higher level of interleukins 1 α , 1 β , 6, 8, and HGF (human growth factor) [25]. There is an interference between the expression of genes that are essential for cell functions and the proinflammatory cytokines. The cytokines might block the expression of these genes, thus damaging the integrity of the cells [27].

The leukocytes can have a negative impact on self-cells by inducing necrosis, apoptosis, and also, by producing Reactive Oxygen Species (ROS) which play a crucial role in sperm activity and physiology. In low concentrations, ROS can induce the hyperactivation of the spermatozoa cells, but in high concentrations, it produces oxidative stress for cells [15, 17]. Catalase, superoxide dismutase, and glutathione protect the cell from ROS in normal con-

ditions. It is known that a high level of ROS can damage the mitochondria by inducing an apoptotic pathway [25].

Conclusion

E. faecalis might play a role in the onset of male infertility, but this role is largely debated in the literature. There is a very thin line between infection, colonization, and contamination, and little is known about the impact of bacteria on spermatozoa. Whereas the patient should receive antibiotics or not, remains a clinical decision, and more research is needed for establishing if there is indeed a correlation between bacterial prostatitis and male infertility.

These cases emphasize the fact that bacterial presence cannot be considered a definitive cause of male infertility, nor be excluded. Given the high unpredictability of the cases, the complexity of the subject exceeds the current knowledge.

To establish the best treatment course, the laboratory results should be correlated with the patient's symptoms. Following the current treatment guidelines and taking into consideration the antibiotic susceptibility test results, the fertility rates might be increased, in some cases.

Authors' contribution

RLC - Data collection; Conceptualization; Writing-original draft; Writing and Editing

CV - Conceptualization; Writing and Editing

BIK - Writing and Editing

ADM - Data collection; Conceptualization

CNC - Writing and Editing; Supervision

Conflict of interest

None to declare.

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