Pyospermia: background and controversies

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Pyospermia (or leukocytospermia) is suspected based on the presence of $>1 \times 10^6$ round cells/mL of ejaculate and diagnosed using peroxidase stain revealing $>1 \times 10^6$ white blood cells/mL. The presence of white blood cells is a concern for overt infections or excessive inflammation, both of which have been postulated to negatively impact bulk semen parameters and fertilization capability. The threshold for pyospermia has been debated upon in the literature, as has the optimal treatment method. In the absence of clinical infectious symptoms, it appears that antibiotics, anti-inflammatory agents, and/or frequent ejaculation may improve bulk semen parameters in men with pyospermia. Further research is needed to adequately assess the effect of these methods on pregnancy and live birth outcomes, especially among couples attempting natural conception compared to those attempting intrauterine insemination or in vitro fertilization. (Fertil Steril Rep® 2021;2:2–6. ©2021 by American Society for Reproductive Medicine.)
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Pyospermia (or leukocytospermia) is a confusing topic with regard to male infertility, fraught with contradictory data of variable quality. Therefore, what is a new practitioner to do when faced with a patient with 5×10^6 or 0.5×10^6 white blood cells (WBCs)/mL ejaculate? This minireview article was written as a primer for the diagnosis, etiology, and treatment of pyospermia specifically for trainees and providers who may not routinely see patients with infertility.

METHODS

A brief review of the literature was performed by searching PubMed using the search terms "pyospermia," "leukocytospermia," "male infertility," and "treatment." In particular, review articles and prospective trials comparing treatment modalities were included. Guidelines from major international societies were reviewed for consensus statements on pyospermia diagnosis and management.

DIAGNOSIS

Approximately 15% of all couples trying to conceive are affected by infertility, and nearly half of these cases are attributable to the male factor. In a standard evaluation by the American Urological Association (AUA), men provided 2 semen samples for analyses, in which several variables are reported: semen volume, pH, sperm concentration, motility, morphology, total motile count, and the concentration of round cells. Pyospermia is suspected if there are over 1×10^6 round cells/mL under a light microscope, but this must be definitively diagnosed using further tests to differentiate WBCs from other similar-looking cells (1).

Round cells are largely categorized as inflammatory or noninflammatory. Inflammatory cells include polymorphonuclear leukocytes, lymphocytes, and macrophages. Noninflammatory cells may include immature germ cells, epithelial cells, or degenerated spermatozoa missing typical identifying features, such as acrosome, midpiece, or tail (2). Although WBCs are thought to play some role in immunosurveillance and the clearance of abnormal sperms, higher concentrations of WBCs induce higher levels of oxidative stress through reactive oxygen species, ultimately damaging spermatozoa motility and fertilization capability (3, 4).

Different stains and methods may be used for semen analysis to differentiate between the inflammatory and noninflammatory cells:

- Peroxidase orthotoluidine blue
- Papanicolaou
- Flow cytometry
- Antibody stains for leukocyte antigens (i.e., CD18, CD45)
- Fluorescence in situ hybridization

The peroxidase test has been recommended by the 2010 World Health Organization (WHO) laboratory manual for the examination and processing of human semen, although it is known to have poor sensitivity (47%–60%) compared with immunocytochemical or flow cytometry methods (>90%)

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(5). AUA, in their 2011 best practice statement, "Optimal Evaluation of the Infertile Male," defined pyospermia as the presence of over 1×10^6 WBCs/mL of semen (6).

Sigman and Lopes (1) established the importance of distinguishing WBCs from the general round cell concentration. In their analysis of 627 infertile men, 9% were found to have $>1 \times 10^6$ round cells/mL, but after immunohistochemical staining for leukocyte surface antigens, only 35% were found to have true pyospermia.

Interestingly, various international governing bodies differ in their approach to pyospermia diagnosis and management. AUA recommends that patients with $>1 \times 10^6$ WBCs/mL semen be evaluated for an underlying genital tract infection or inflammation (6). However, there are no formal recommendations on how patients should be evaluated (i.e., based on symptoms, urine culture, semen culture, etc.) or on the type or duration of treatment. The Canadian Urological Association does not reference pyospermia in their guideline, "Workup of azoospermic males" but rather references it in the "Prostatitis" guideline, in which semen culture is not recommended (7).

In their 2016 update, the European Association of Urology also noted that it is unclear what impact urethritis, prostatitis, orchitis, and epididymitis have on sperm quality and overall fertility. WBCs in the semen may be a marker of inflammation rather than a sign of an underlying bacterial or viral infection, as previously noted. Semen culture may be useful in the diagnosis of symptomatic prostatitis. However, although antibiotic treatment for prostatitis is the best course to relieve symptoms, it is not clear whether this will also improve the probability of conception. Other treatment options may include anti-inflammatory agents, surgery to normalize urine flow, and physical therapy. In a setting of elevated numbers of round cells, the European Association of Urology has recommended peroxidase staining to differentiate WBCs from noninflammatory round cells because resultant inflammatory cytokines may influence sperm function (8).

However, the threshold for pyospermia has been debated upon in the literature. Higher numbers of WBCs in the ejaculate have been shown to be positively correlated with higher incidences of acrosomal damage and midpiece and tail deformities in a morphological analysis (9). Average reactive oxygen species production was found to be 77 times greater in samples with over 0.1×10^6 WBCs/mL, suggesting that the WHO's threshold of 1×10^6 WBCs/mL is far too high (10). Punab et al. (11) compared semen cultures from patients with chronic pelvic pain syndrome with those from patients presenting for infertility evaluation and those from men whose partners had chronic gynecologic infections. All 3 groups demonstrated a positive correlation between WBC count and the number of isolated microorganisms. As sperm agglutination occurs at approximately 10,000 microorganisms/mL, this was considered the threshold for significant bacteriospermia. A receiver-operating characteristic curve analysis has shown that a cutoff of $>1 \times 10^6$ WBCs/mL has a sensitivity of only 23% for identifying significant bacteriospermia and that a more optimal threshold may actually be 0.2×10^6 WBCs/mL (11).

ETIOLOGY

A difficulty with establishing a threshold for pyospermia is the uncertainty of whether the presence of WBCs in the semen is indicative of a true infection or an inflammatory process.

Noninfectious causes may include the following (12):

- Toxins: environmental, tobacco, alcohol, or marijuana
- Varicocele
- Autoimmune disorders
- Poor sperm viability
- Chronic prostatitis
- Congenital genitourinary malformations, such as posterior urethral valves (13)

Specific culture methods are required to identify genitourinary pathogens, which may exclude other organisms not easily detected using standard aerobic and anaerobic cultures. It is also possible that there are supportive bacteria in the semen that enhance sperm function and health, much like healthy vaginal flora.

The relationship between the presence of bacteria and WBCs in the semen is also questionable. In a retrospective review of 7,852 semen analyses from men referred to an infertility center, Domes et al. (12) found a bacteriospermia incidence of 15% and a pyospermia incidence of 19%. The investigators found that the presence of bacteriospermia had no significant impact on sperm concentration, morphology, or motility. Furthermore, there was no statistically significant correlation between bacteriospermia and pyospermia, which the investigators defined as ≥ 1 polymorphonuclear neutrophils per 100 sperms. At this pyospermia threshold, the investigators did note a significant reduction in sperm concentration, morphology, and motility. These findings were supported by those of Filipiak et al. (14), who found no significant difference in the bulk semen parameters between men with or without bacteriospermia, which they defined as >10,000 colony-forming units/mL of ejaculate. Although approximately 70% of their population had bacteriospermia, only 6 patients met the WHO's criteria for pyospermia, 4 of whom did not have significant bacterial colonies in the semen culture.

Although the concentration of WBCs may not be a reliable predictor of seminal bacteria, the presence of bacteria in the ejaculate represents a new frontier for research, possibly using next-generation sequencing (NGS). NGS most commonly employs a 16S ribosomal ribonucleic acid analysis to extract large-scale information about microbial genetics, identifying organisms that are not otherwise easily cultured. Weng et al. (15) investigated the relationship between seminal bacterial communities identified by NGS and semen quality in 96 subinfertile men. Their results suggested that it is not the mere presence of seminal bacteria but rather the proportion of "healthy" bacteria, such as *Lactobacillus*, that impacts the semen parameters, much like vaginal or fecal flora.

More research is needed to establish the relationship between seminal plasma microorganisms and fertility. This has the potential to explore new treatment options for male infertility because standardized management recommendations for pyospermia are lacking.

MANAGEMENT

Management strategies greatly differ for men with infertility as well as asymptomatic pyospermia. The strategies are categorized into antibiotics or anti-inflammatory agents and are largely centered on the hypotheses that WBC presence is secondary to either bacterial infection or excessive inflammation or both.

Antibiotics

Brunner et al. (16) conducted a literature review of 11 studies, evaluating pyospermia therapies and their outcomes. In 3 papers, significant resolution of pyospermia was observed in patients treated with antibiotics compared with that observed in controls, and in 3, no difference was found. One study randomized patients to 4 pyospermia treatment groups: doxycycline (100 mg twice daily for the first week, then 100 mg daily for the following 3 weeks), placebo, frequent ejaculation (at least every 3 days), and doxycycline plus frequent ejaculation. The investigators found that the combination therapy of antibiotic and frequent ejaculation was the most successful, with the effects persisting at 3-month follow-up (17). Ten pregnancies were achieved, all in couples whose male partners experienced pyospermia resolution. Frequent ejaculation was thought to clear stored secretions, which may facilitate antibiotic effectiveness and minimize inflammation. Yamamoto et al. (18) conducted a similar placebo-controlled study using trimethoprim-sulfamethoxazole 2 times per day for a month with and without frequent ejaculation. Seventy-six percent of men in the combination therapy group experienced resolution of their pyospermia compared with 56% of the men in the antibiotic-alone group and 6.7% of the men in the placebo group. In contrast, Hamada et al. (19) found no statistically significant improvement in the semen parameters in men with infertility treated with doxycycline (100 mg twice daily for 3 weeks). This could be attributed to their definition of pyospermia, which was $\geq 0.2 \times 10^6$ WBCs/mL. Interestingly, the pregnancy rate was higher in patients receiving treatment compared with that in untreated controls (47% vs. 20%, respectively).

In summary, the most common antibiotic regimens used in the literature are as follows:

- Doxycycline (100 mg daily for 3–4 weeks)
- Trimethoprim-sulfamethoxazole (80 mg/400 mg twice daily for 4 weeks)
- The abovementioned combination with or without frequent ejaculation (every 3 days)

Anti-Inflammatory Agents

As pyospermia is also thought to be secondary to inflammation rather than to infection, anti-inflammatory agents have also been investigated. Oliva and Multigner (20) studied the effect of ketotifen (1 mg twice daily for 3 months), an antihistamine that stabilizes mast cells in men with pyospermia and infertility. The study was greatly limited by the lack of a placebo-controlled group, but the investigators found improvement, if not resolution, in pyospermia at the fourth week of the treatment. Sperm motility and morphology were the only bulk semen parameters to improve from a median of 42% and 35% to 55% and 44% at 8 weeks, respectively. Of 55 patients, 16 achieved pregnancy within 6 months of ketotifen initiation.

Cyclooxygenase-2 inhibitors are a class of nonsteroidal anti-inflammatory drugs that ultimately block prostaglandin secretion, decreasing inflammation. Gambera et al. (21) administered 25 mg of rofecoxib once daily for 30 days to 47 men with pyospermia. Progressive motility doubled from 23.3% to 46.9%, morphology increased from 19% to 34%, and pyospermia resolved. There were no reported side effects. Three pregnancies were conceived naturally and 7 via intrauterine insemination (performed 6–8 weeks after the therapy). Lackner et al. (22) prescribed valdecoxib (20 mg daily) to 12 patients with pyospermia. Although semen analyses 3 months later showed incomplete pyospermia resolution, the average sperm concentration doubled from 22.5×10^6/mL to 48×10^6/mL, and the morphology and motility increased from 20% to 36% and from 28% to 33.5%, respectively. Both the studies were limited by the lack of placebo-controlled groups and small study sizes.

In summary, the most common anti-inflammatory regimens used in the literature are as follows:

- Antihistamine: ketotifen (1 mg twice daily for 3 months)
- Nonsteroidal anti-inflammatory drugs: rofecoxib (25 mg daily for 1 month) or valdecoxib (20 mg daily for 2 weeks)

Many questions have been left unanswered by the available literature. How long do patients require treatment for? Are the effects lasting, or is it possible that patients may require multiple rounds of treatment? Although bulk semen parameters appear to improve with various treatments, what is the ultimate effect on pregnancy and live birth rates? Is this altered by assisted reproductive technology?

IMPACT ON ASSISTED REPRODUCTIVE TECHNOLOGIES

This final question has proven very difficult to answer based on the available literature. A multivariate analysis by Ricci et al. (23) revealed that there was no difference in the fertilization or cleavage rates in 164 couples with and without pyospermia who underwent in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) even after adjusting the pyospermia cutoff from 0.2×10^6 WBCs/mL to 2×10^6 WBCs/mL. These results call into question the need for any pyospermia treatment if the couple is scheduled for IVF. However, an earlier study by Yilmaz et al. (3) found that couples with $>1 \times 10^6$ WBCs/mL who received ICSI had poorer fertilization rates and embryo development compared with couples with $<1 \times 10^6$ WBCs/mL. Interestingly, in this study, the pregnancy rates were unaffected. To

further complicate the matter, in a 7-year study of over 1,900 couples who underwent IVF with or without ICSI, higher fertilization, cleavage, and pregnancy rates were found in couples with higher concentrations of WBCs (4). The only negative effect seen in the pyospermia group was higher rates of early pregnancy loss and ectopic pregnancy.

CURRENT MANAGEMENT STRATEGY

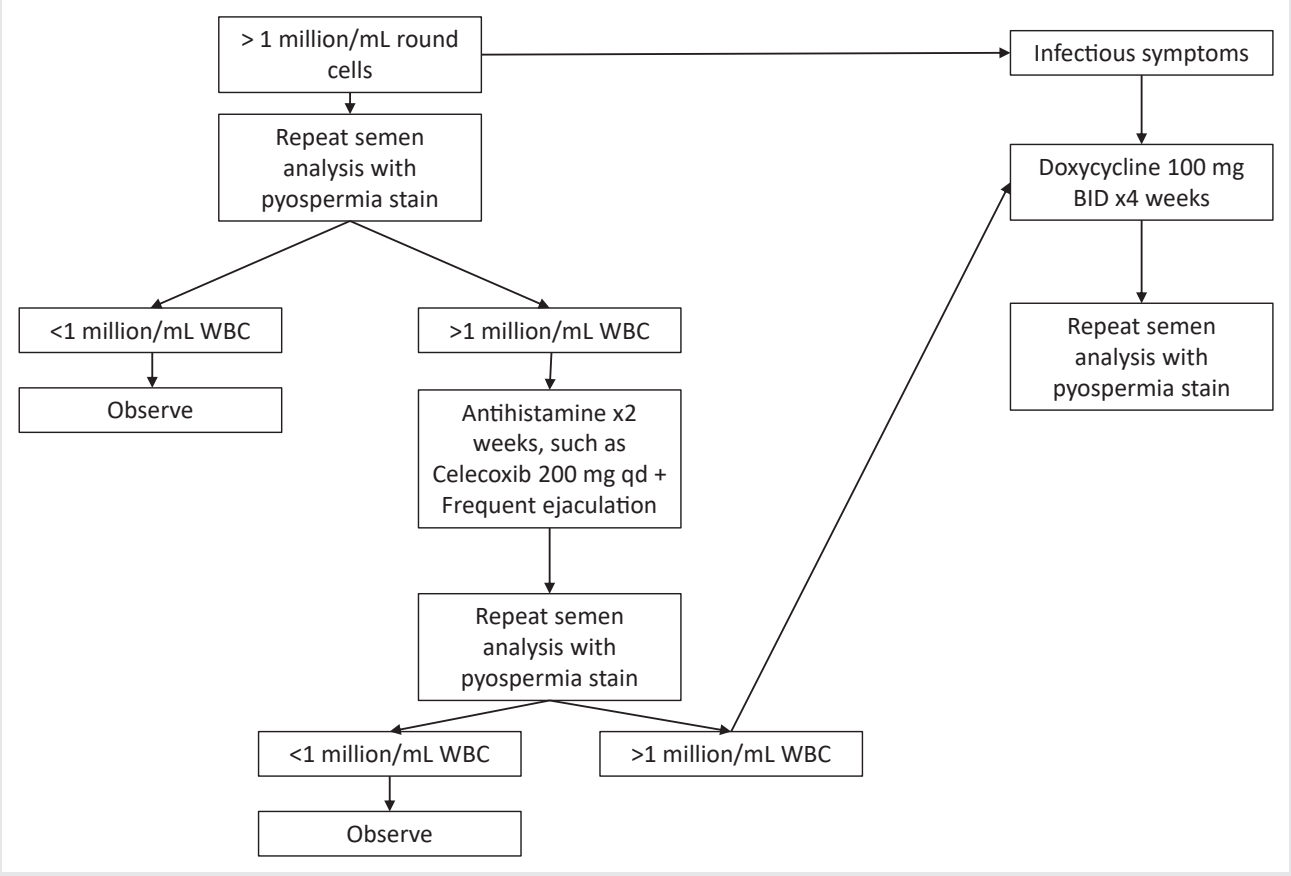
Our diagnosis and treatment algorithms at the University of Illinois at Chicago are shown in Figure 1. Our infertility specialists prescribe pyospermia staining (Papanicolaou stain) for men presenting with $>1 \times 10^6$ round cells/mL in their initial semen analysis. Treatment is recommended for men with infectious symptoms (pelvic pain, dysuria, painful ejaculation, etc.) and/or $>1 \times 10^6$ WBCs/mL revealed in the pyospermia staining. Men with infectious symptoms are prescribed doxycycline (100 mg 2 times per day) for 4 weeks, after which a repeat semen analysis with the pyospermia stain is performed. Men with $>1 \times 10^6$ WBCs/mL revealed in the pyospermia staining but with no symptoms of an acute infection may be started on an anti-inflammatory agent, such as celecoxib (200 mg daily for 2 weeks), and frequent ejaculation (every 2–3 days). A repeat semen analysis with pyospermia staining is then performed, and if there are persistent WBC concentrations over 1 million/mL, a course of doxycycline (100 mg 2 times per day for 4 weeks) is prescribed.

As a part of the routine infertility evaluation, all men undergo a physical examination for varicocele. An extensive history is also taken to assess for lifestyle factors that may contribute to pyospermia, including smoking, marijuana use, excessive alcohol intake, illicit drug use, or the use of hot tubs, saunas, etc. Patients are counseled to discontinue or at least minimize the use of these factors when fertility is desired.

Patients who are already scheduled for IVF still undergo specialized pyospermia staining, although they are counseled that the reproductive outcomes do not appear to be universally affected by the presence of WBCs in the ejaculate.

In conclusion, pyospermia is heralded by the presence of $>1 \times 10^6$ round cells/mL. It is most commonly diagnosed using peroxidase stain and is defined by the presence of $>1 \times 10^6$ WBCs/mL. Treatment with antibiotics is certainly indicated in patients with infectious symptoms. However, in the

FIGURE 1



Pyospermia diagnosis and treatment algorithm.

Velez. Pyospermia in male infertility: a review. Fertil Steril Rep 2021.

absence of symptoms, it is unclear whether pyospermia is an indicator of subclinical inflammation or infection or is possibly a nonpathologic finding. Different treatment options have been investigated, including antibiotics, anti-inflammatory agents, and frequent ejaculation, with varying effects on bulk semen parameters. Further research is needed to adequately assess the effect of these management methods on pregnancy outcomes, especially among couples attempting natural conception compared to those attempting intrauterine insemination or IVF.

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