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·Review·

Current perspectives on pyospermia: a review

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Abstract

Pyospermia is an abnormal laboratory finding of high concentration of white blood cells in human ejaculates during infertility workup. The role of pyospermia and its impact on fertility is an important consideration in the management of infertility. Etiology, pathogenesis, diagnostic modalities and the management of pyospermia are reviewed in this paper. Current use of antibiotics and the intrinsic production of antioxidants in the management of pyospermia are also discussed in this review. *(Asian J Androl 2007 Sep; 9: 593–600)*

Keywords: pyospermia; semen; leukocytes; ejaculate; infertility

1 Introduction

Pyospermia is a laboratory finding categorized as the abnormal presence of leukocytes in human ejaculates. It is presumed to be a clinical sign of infection/inflammation of the accessory sex organs or of the lower genitourinary tract [1–3]. Numerous studies demonstrated that these leukocytes present in ejaculates have a physiological effect on sperm functions, which may further implicate male infertility [4–7]. Berger *et al*. [8] suggested that the presence of leukocytes in seminal fluid ejaculate would be the best indicator of an atypical sperm penetration assay (SPA) in normal semen analysis. Studies by Maruyama *et al*. [9] reported decreased fertilizing ability of the donors' sperms after adding the supernatant of white blood cells (WBCs) to a fertile donors' semen.

Wolff *et al*. [7] demonstrated a strong inverse relationship between major seminal fluid parameters and symptomatic pyospermia. The seminal fluid parameters currently studied include: total sperm count, percent motility and morphology, sperm velocity, presence of fructose and the total number of motile sperm**.** If the semen analysis contains more than 10^6/mL of WBCs, microbiological evaluations such as the culture of urine and ejaculates are essential to determine if accessory sex gland and lower urinary tract infections are present.

Current diagnostic modalities on determining the concentrations of WBCs in semen focus on sperm functions. The impact of leukocytes depends upon the stages and sites at which WBCs enter the semen, the involvement of specific types and concentrations of leukocytes, and their states of activation. Berger *et al*. [8] showed that the presence of one neutrophil in a pool of 100 sperms is sufficient to increase the risk of an abnormal SPA by a factor of 8.17. However, studies on temporal morphology, transition of leukocytes and its relationship to sperm parameters are still in the early stage and are not being rou-

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tinely used in laboratory diagnosis of pyospermia because of such factors as time and cost.

2 Historical perspectives

Pyospermia is established when the concentration of seminal WBCs is in the range between 5×10^5/mL and 5×10^6/mL of seminal fluid during semen analysis [10]. Pyospermia is also defined when more than 1 000 000 WBCs are counted in 1 mL of semen [11]. Shy *et al*. [12] suggested that pyospermia is present when six or more WBCs are present among 100 spermatozoa. The World Health Organization (WHO) internationally standardizes and defines pyospermia as 1×10^6 WBCs/mL semen as diagnosed by measuring either peroxidase or by immunohistological detection methods [13]. This is currently the universally accepted definition of pyospermia. It establishes a universal guideline to determine the number of WBCs in semen that may have an impact on male fertility.

Historically, the laboratory discrepancies of WBC count in semen can be explained by recent discoveries of variable types of immature germ cells that are wrongly identified as WBCs [14]. These immature cells closely resemble WBCs in size, morphology and the diverse WBC types distinguished by different laboratory methods. When pyospermia is present, the determination of the actual concentration of WBCs in semen will be difficult because of the erroneous presence of WBCs [14]. "Pseudo-pyospermia" is the clinical terminology applied when diagnosis of "pyospermia" is mistaken by the presence of immature germ cells, rather than the actual WBCs in semen. Fortunately, special immunohistochemical staining can differentiate immature germ cells from WBCs and can establish an accurate leukocyte count in SPA [14]. This staining technique is mainly time consuming and expensive, and hence cannot be routinely used during semen analysis for the diagnosis of pyospermia.

3 Etiology

Pyospermia has multifactorial causes, including infection, inflammation and autoimmunity [2]. The etiology can be classified into several categorie: presence of defective sperm, varicocele and chronic prostatitis, smoking, drug abuse like marijuana (social causes), alcohol, exposure to irritants and toxins, use of vaginal products by partner during sexual activities, abstinence, vasovasostomy, clomiphene citrate therapy, and urethroplasty, *Chlamydia Trachomatis*, *Gardnerella vaginalis* and *Ureaplasma urealyticum*, in patients' sexual partners (genital infection), lower CD^{4+} cell counts in HIV patients. Apart from the diverse etiologies, many other causes have been drawn from various clinical researches in recent years. In 1995, Matthews *et al*. [15] described that clomiphene citrate-treated men (over the age of 35) are more likely to develop pyospermia when they are treated with non-bacterial drug therapy, which may adversely impact male fertility. Anderson [10] suggested that pyospermia is associated with subclinical genital tract infection. However, current research studies failed to recognize any specific bacterial pathogens in semen that are responsible for the disease. In addition, with the widespread prevalence of AIDS, a potential association between a low CD4+ cell count in HIV patients with pyospermia and other presentations of sperm abnormalities has been reported [16].

4 Pathogenesis

To understand the pathological pathways of pyospermia, the exact roles and types of WBCs should be delineated. There are three main categories of WBCs that can be morphologically distinguished: granulocytes, monocytes and lymphocytes. Granulocytes can be further subdivided into neutrophils, eosinophils and basophils. Under a normal human defensive response to invasion by foreign matters, WBCs would accumulate in the infected area. Neutrophil, which is the predominant granulocyte subtype, specifically invades the lipid membrane of the pathogens by releasing reactive oxygen species (ROS).

ROS is the specific class of oxygen free radicals that are responsible for damaging the lipid components of the sperm membrane [17]. ROS is the highly reactive oxidizing agents that are also produced by defective spermatozoa *in vivo* in addition to the granulocytes [18–20]. ROS is also produced by the sperm from a variety of diseases such as vasectomy reversal, varicocele and idiopathic infertility, besides the leukocytes [21]. Hydrogen peroxide (H_2O_2) is the most potent species among all types of ROS. There are also other species of ROS such as nitric oxide radicals (NO-), peroxynitrite anion ($ONOO^-$), superoxide (O^{2-}), and hydroxyl (OH^-) that exhibit the same effects [20, 22, 23].

Spermatozoa contain the genetic material and are sur-

rounded by a double-layered membrane. This membrane is composed of lipid and protein just like the normal plasma membrane. The lipid portion of this membrane consists of a special phospholipid, Plasmalogen, and a substantial concentration of polyunsaturated fatty acids (PUFA), which play an important role in the development of pyospermia. When spermatozoa are invaded by the surrounding ROS (oxidative stress), the lipids that are exposed on the sperm's membrane become the specific targeting sites of ROS [20, 24].

Intrinsically, the pathophysiological pathway of ROS-induced deleterious spermatozoa effects can be explained through a mechanism named lipid peroxidation (LPO) [25]. In any event in response to infection, inflammatory, or autoimmune activities, ROS production by WBCs will be triggered through the LPO mechanism. LPO is initiated when the sperm membrane is attacked by ROS. After the successful entrance through the spermatic membranes, ROS will proceed to penetrate further where all the genetic materials are located. On the molecular level, once ROS invade the sperm, they destroy the mitochondrial DNA and thus disrupt the intracellular ATP production in spermatozoa [24, 26]. With the decreased production of ATP, energy for the sperm activities, motility and other functions will be lost.

The modulation of ROS production in seminal plasma is equilibrated between the pro-oxidant and anti-oxidant activities [24, 27]. Pro-oxidant activity (pro-infertility), which is sperm hostile, is the action that generates ROS, which decreases sperm activity and function through the LPO mechanism. Anti-oxidant activity (pro-fertility) is sperm friendly and is the action that scavenges the ROS. This activity is maintained by the presence of a significant level of antioxidants, such as the enzyme superoxide dismutase (SOD), catalase, urate, sulphydryl groups, tocopherol (vitamin E), vitamin C (ascorbate) and carotenoids [27–30]. These anti-oxidants directly target the ROS activities or LPO mechanism. They protect the sperm membrane and spermatic DNA structures and prevent them from destruction under oxidative stress triggered by ROS [28].

Glutathione peroxidase (GSH) is another antioxidant enzyme, which specifically acts on eliminating various ROS such as hydrogen peroxides. While undergoing this activity, GSH is converted into its inactive form, glutathione disulfide (GSSG). In order to resume its active form, NADPH is needed. One of the modern diagnostic modalities in pyospermia is to determine the GSH/GSSG ratio. The high ratio indicates that more GSH is present *in vivo* to counteract the peroxidase activities by the peroxides [31, 32].

Without the inhibitory presence of ROS, sperm resumes its normal activities. A positive oxidative stress status (OSS) is the term used to denote the increased level of ROS production towards pro-oxidants in semen and the decrease in antioxidant activities, the antagonist of ROS and vice versa [33]. The balancing mechanisms between pro- and anti-oxidants in regulating the effect of WBCs on sperm are presented in Figure 1.

Besides the contribution of positive OSS by the ROS activities, Sikka [32] reported that production of chemokines such as IL-8 and GROα, in response to infection and inflammation, may also contribute to the positive oxidative stress status and further decrease motility and other functions of sperms and thus categorize them as the pro-inflammatory substances. However, IL-10 is the other chemokine that acts as anti-inflammatory substance. The two types of chemokines, like the balances between ROS and SOD, demonstrate the counterbalance phenomenon for the determination of disease states.

5 Investigations

Pyospermia is considered to be one of the causes of male infertility [34]. Shy *et al*. [12] demonstrated an association between the presence of leukocytes in semen and the subsequent decreased pregnancy rates. With these statistical data in mind, investigations should be comprehensive. A current clinical investigation should ideally include determination of the couple's history of infertility, their sexual habits, the spouse's primary and secondary sexual characteristics, and medical, genetic, surgical, family history, exposure to gonadotoxins and a thorough review of systems. Laboratory testings include differential sperm separation method, endocrine evaluation, semen analysis, and if indicated, culture of the ejaculates, quantitation of leukocytes in semen, antisperm antibodies and immature germ cells in semen [4, 35, 36]. An important factor to be considered is that the short half life of polymorphonuclear neutrophils (PMN) in semen makes them a major source for factors that can be harmful to sperms. PMN have also been shown to be activated by sperm cells resulting in formation of neutrophil extracellular traps that trap sperm [24].

Evaluation of pyospermic patients comprises a focused history and physical examination, examination of

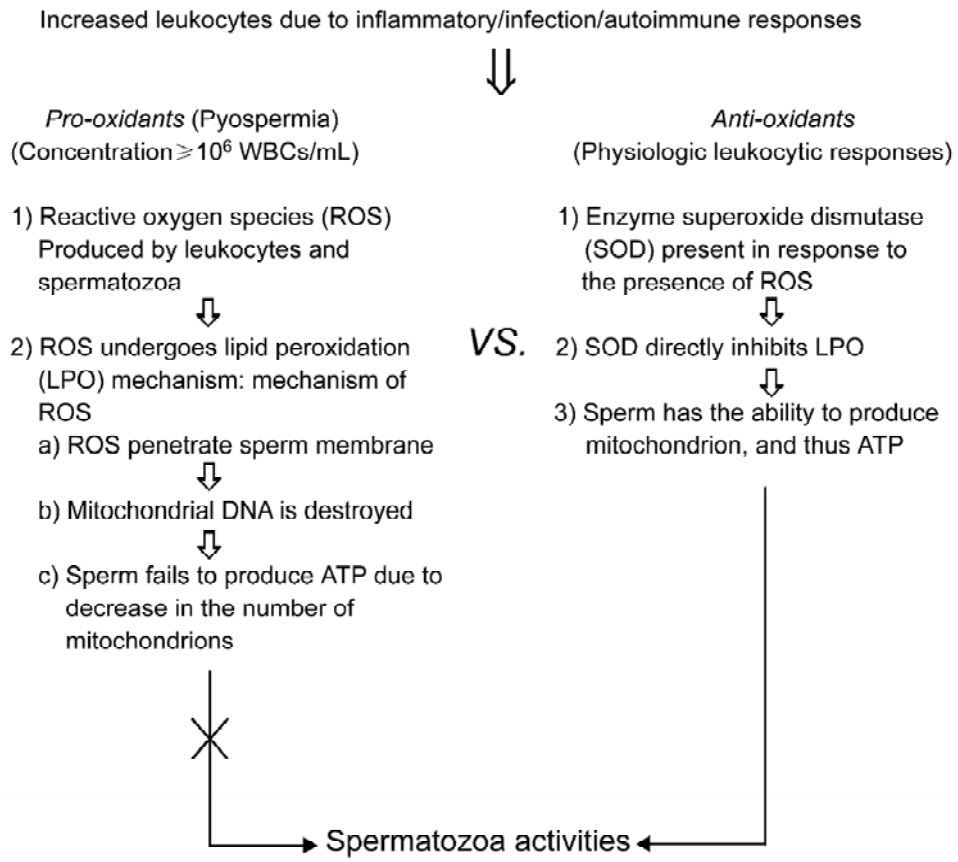

Figure 1. The effect of white blood cells (WBCs) on spermatozoa activities. The scheme demonstrates the balancing mechanisms between pro- and anti-oxidants.

prostatic fluid, semen analysis and ultrasound imaging of the accessory sex glands, ejaculatory duct, and lower urinary tract. There are various techniques for detection of pyospermia in human semen. Persistent pyospermia, which is pyospermia observed in semen specimens collected at 3-month interval, is an indication for repeat physical and microbiological examination of semen after which empiric antibiotic treatment can be initiated [37].

Immounocytological method is the most recent laboratory technique that uses monoclonal antibodies as labels and is the best method for distinguishing leukocytes in semen. This method, however, is too expensive and time-consuming to be used on a routine basis. It is rather more applicable in a research setting. The Bryan-Leishman stain is used in many studies and is a useful method of identifying total leukocytes per 100 sperm. The number of WBCs per 100 sperm is usually counted on a direct semen smear stained by the Bryan-Leishman method of Couture and it also allows differentiating leukocytes from immature sperm cells [38]. But this stain is not used widely because it takes 2–2.5 h, and requires about 20–30 min to accurately prepare multiple slides for examination. This technique of measuring leukocytes per 100 sperm gives accurate measurements when sperm counts are normal but overestimates leukocyte numbers in oligozoospermic men. Monoclonal antibodies facilitate accurate counts but are time-consuming and expensive to be used on a routine basis.

Shekarriz *et al.* [39] in their evaluation of semen by Endtz test showed the presence of irregular quantity of ROS accumulation in semen, so as to indicate the presence of pyospermia. The level of elastase present in seminal plasma has been shown to bear a direct relationship to pyospermia [40]. Elastase is an enzyme acting as distinct marker of inflammation. Its level correlates with the amount of WBC presented in semen, so that the de-

gree of inflammation can be ascertained [40]. IL-6 and granulocyte elastase are also found to be useful and suitable markers for silent genital tract inflammation [41].

6 Differential diagnosis

The differential diagnosis of symptomatic pyospermia includes infection, autoimmune disease, and inflammation of accessory sex glands and lower male urogenital tract. Urogenital infections include acute and chronic prostatitis, seminal vesiculitis, epididymo-orchitis, cystitis, urethritis, urethral stricture, stone disease, foreign bodies, upper urinary tract infection, retrograde ejaculation, and localized sepsis of the adjacent lower gastro-intestinal tract and asymptomatic bacteriuria. The chronic infections that may result in pyospermia include fungal, mycobacterial, congenital lesions causing infection of the urogenital tract. Refractory autoimmune diseases that afflict the urogenital tract include Behcet's syndrome and Reiter's disease.

7 Management

There is no definite medical management of pyospermia because the specific causes of the disease cannot be isolated (Figure 2). The current management methodology surrounds elimination of the cause (if any), correction of predisposing factors, elimination of infection and protection from free radicals and oxidative agents produced inside the body as a result of inflammation, infection, or auto-immunity. The treatment options can be categorized into antibiotics treatment (doxycycline, trimethoprim and sulfamethoxazole, ofloxacin), medications such as calcium dobesilate, propofol, rebamipide, N-acetyl-L-Cysteine (NAC), glutathione, coenzyme ubiquinol-10 (CoQ10), ferulic acid (FA), and vitamin C and E (α-tocopherol). Other alternative treatments include Chinese herbs (Magnolol), natural product antioxidants, and the practice of frequent ejaculation [42–49].

The removal of cause and primary predisposing factors include the correction of any congenital or acquired defect in the genitourinary tract harboring infection and inflammation, vesicourethral reflux, prostatic obstruction and infection, retrograde ejaculation, and urethral valves. These modalities follow the mechanism that scavenges ROS with the inhibition of LPO, such as coenzymes ubiquinol-10 [50, 51].

Several clinical trials have investigated the efficacy of antibiotic therapy to treat patients with pyospermia in an attempt to improve fertility. Many studies have reported a decrease in seminal WBC concentration and improved sperm function following a course of antibiotics [52–54]. However, large clinical trials reported no significant beneficial effect of either doxycycline or Bactrim therapy because of a high rate of spontaneous resolution in untreated leuko-cytospermic men [55, 56]. By studying the efficacy of doxycycline and the combination of doxycycline with ceftriaxone for the treatment of asymptomatic men with pyospermia, it was disco-

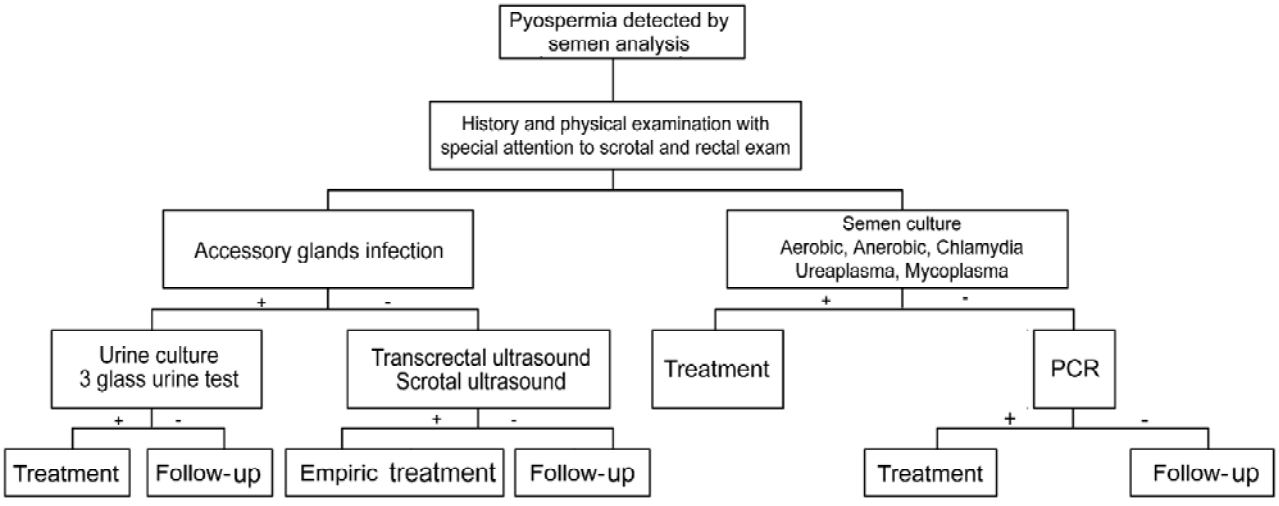

Figure 2. Evaluation of patients with pyospermia.

vered that antibiotic therapy is not beneficial for asymptomatic men with pyospermia [57]. Because of the risk factors associated with antibiotic therapy like their relative toxicity and the mechanisms by which antibiotics affect spermatogenesis and spermatozoa function [58], it would be important in the future to design a study that includes a WBC enumeration assay, which will allow one to differentiate between chronic and acute pyospermia and positively diagnose bacterial infections by culture or molecular biology technique before the initiation of therapy.

Other therapies have been proposed for the treatment of genital tract inflammation associated with pyospermia. Clinical trials are presently underway with vitamin E (α-tocopherol) which acts as an anti-oxidant in patients with a high concentration of free radical levels in their semen by reducing the lipid peroxidation activities *in vitro* in human spermatozoa [59–62]. Recently Akiyama [63] reported that the administration of ethylcysteine and tocopherol (vitamin E) resulted in significant improvement in sperm function but there was no statistically significant effect on sperm density or motility. The author also reported significantly reduced level of ROS after the administration of ethylcysteine [63]. In addition, pentoxifylline has been used recently to enhance sperm motility in severely oligospermic and asthenospermic men [64, 65]. It decreases ROS generation by spermatozoa and thus increases the anti-oxidant activities *in vitro* [64, 66]. Other treatments, such as administering glutathione *in vivo*, showed improvement in the function of spermatozoa [67]. Because there are no multi-institutional fertility studies, it is unknown if male infertility can be improved. The diagnosis of pyospermia is usually based on the levels of leukocytes in semen but controversy remains over the minimum leukocyte level that impairs fertility. ROS can be found even in patients with very low seminal WBC counts and rises with an increase in WBC count. Therefore, it is relatively difficult to determine a safe minimum WBC count (as per WHO guidelines) as the presence of any WBC is associated with ROS, which damages semen quality and may therefore impair fertility [68].

8 Conclusion

Further researches are needed in this area because the methods for enumerating leukocytes and characterizing their products are not standardized. Also, the exact role of WBCs, their products and subtypes (i.e. neutrophils, lymphocytes, monocytes, eosinophils and basophils) in the male reproductive tract, is not clear. Moreover, the exact role of infections, especially sexually transmitted viral infections of the male genital tract, in causing pyospermia is unknown. There have been significant advances in the medical treatment of pyospermia in recent years [69–72], especially that with anti-oxidant agents. Further research is needed to explore the efficacy of various anti-viral, immunosuppressive and current anti-inflammatory drugs in the treatment and prevention of pyospermia. So far, evidence seems to indicate that progress has been made with regard to the understanding of pathophysiological mechanisms involved in damaging spermatozoa in patients with significantly high amounts of leukocytes in the semen. Nonetheless, research should continue to exactly identify the intensity and nature of damage caused by various leukocytic subtypes and their products.

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