

# Laboratory and clinical management of leukocytospermia and hematospermia: a review

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Ther Adv Reprod Health

2020, Vol. 14: 1–12

DOI: 10.1177/  
2633494120922511

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**Abstract:** Leukocytospermia and hematospermia are defined as the presence of abnormally high white blood cell and red blood cell concentration in the semen, respectively. Numerous etiologies and various implications on fertility have been identified. In a small proportion of men, the presence of white blood cells or red blood cells can adversely affect sperm quality by the production of reactive oxygen species. Several methods have been used to assess the presence of white blood cells and red blood cells in samples, such as identification of round cells, immunohistochemical staining using monoclonal antibodies, the Endtz test, the peroxidase test, and flow cytometry or microscopy. In addition, techniques have been identified to separate sperm samples from white blood cells and red blood cells for cryopreservation to improve outcomes in assisted reproductive technology. In this review, laboratory and clinical management of leukocytospermia and hematospermia are discussed. Currently available diagnostic methods and treatment options are outlined, and available optimal cryopreservation techniques for samples with white blood cells or red blood cells are summarized.

**Keywords:** cryopreservation, hematospermia, leukocytospermia, management, sperm separation, testicular biopsy

Received: 30 January 2020; revised manuscript accepted: 8 April 2020.

## Introduction

Some semen samples contain excess white blood cells (WBCs; leukocytospermia) or excess red blood cells (RBCs; hematospermia), both of which can adversely affect sperm quality and fertility potential.<sup>1,2</sup> Leukocytospermia is a condition in which there is abnormally high concentration of WBCs within the semen.<sup>3</sup> Leukocytospermia has been shown to be a negative prognostic factor for fertility,<sup>4</sup> and etiology can be from a wide variety of sources. Current methods for detecting leukocytospermia include identification of round cells, immunohistochemical staining using monoclonal antibodies, the Endtz test, the peroxidase test, and flow cytometry.<sup>5,6</sup> Studies have evaluated the use of antibiotics, anti-inflammatory agents, and anti-oxidants as treatment; however, results have been varied, and controversy remains. On the contrary, hematospermia is defined by the presence of

RBCs in the ejaculate.<sup>7,8</sup> It has been shown that the presence of RBCs in the semen can affect the fertilization potential of spermatozoa.<sup>9</sup> It is commonly diagnosed by macroscopic identification in the ejaculate or can be commonly seen microscopically in semen analysis. Most cases have no serious cause and will spontaneously resolve; however, a more thorough urological workup is indicated to identify an underlying cause if symptoms persist. Upon diagnosis of either of these conditions, a variety of methods exist to separate WBCs or RBCs for cryopreservation or use in assisted reproductive technology (ART).

Sperm cryopreservation is used for a variety of purposes, including sperm donation by healthy men and fertility preservation in men with medical conditions such as cancer that may impair future fertility. One goal of cryopreservation is to retain

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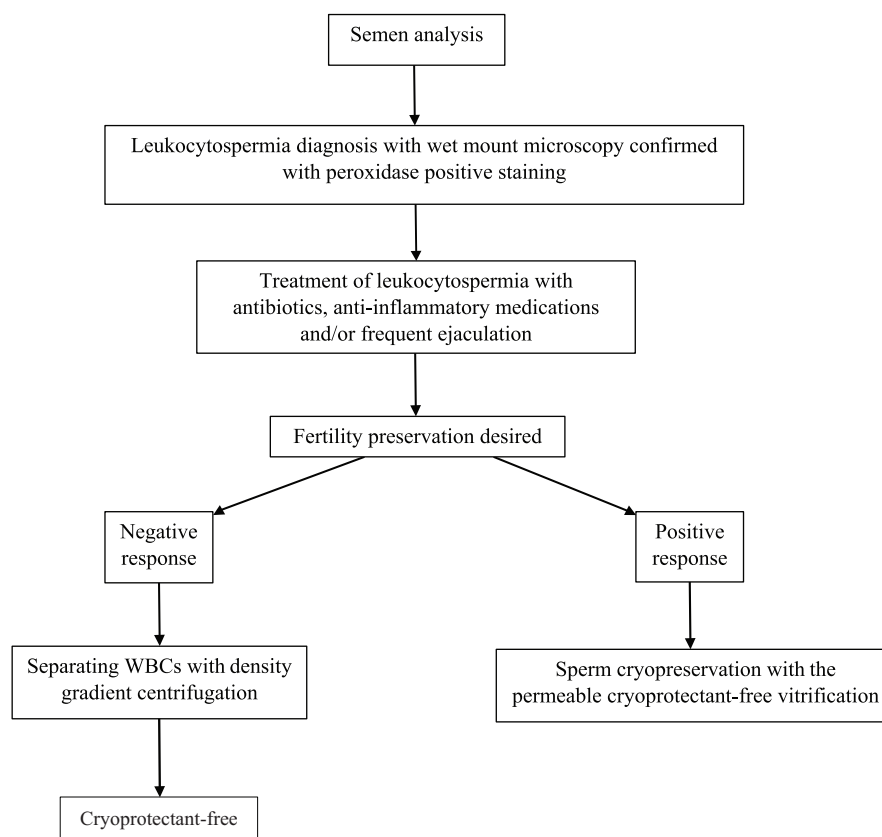
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**Figure 1.** Algorithm for management of semen samples obtained from leukocytospermic infertile males.

sperm parameters for use in future fertility procedures. However, cryopreservation along with RBCs or WBCs can cause harmful alterations in the structure and function of spermatozoa.<sup>10</sup> The purpose of this review is to outline the laboratory and clinical management of leukocytospermia and hemospermia and discuss the currently available cryopreservation techniques.

### Leukocytospermia

Leukocytes can be found throughout the male reproductive system. Leukocytes in semen originate mostly from the epididymis, where they play an important role in immunosurveillance, including phagocytic clearance of abnormal sperm. The most common type of leukocytes in semen are granulocytes (50–60%), followed by macrophages (20–30%) and T-lymphocytes (2–5%).<sup>11</sup>

Leukocytospermia (also termed leukospermia or pyospermia) is a condition in which there is an

abnormally high concentration of WBCs in the semen. In healthy men, WBCs are usually found in small amounts in semen samples.<sup>12</sup> The World Health Organization (WHO) defines leukocytospermia as  $>1 \times 10^6$  WBCs/mL in a semen sample.<sup>3</sup> Leukocytospermia is considered an inflammatory disease. In most cases, the inflammatory syndrome is secondary to a urogenital bacterial disorder. However, other conditions may also lead to leukocytospermia, including viral infections, varicocele, smoking, or trauma such as spinal cord injury.<sup>6</sup> Thirty percent of infertile males have leukocytospermia, although in 80% of leukocytospermic infertile males, no microbial infection can be detected in their semen.<sup>13</sup> An overview of clinical and laboratory management of leukocytospermia can be seen in Figure 1.

### Leukocytospermia and infertility

Leukocytospermia is found more often in infertile men than in fertile men.<sup>14</sup> However, the

relationship between the condition and sperm quality remains controversial. Some studies have shown leukocytospermia to be a negative prognostic factor for fertility,<sup>4</sup> and the reasons are multifactorial. For example, leukocytospermia can impair spermatogenesis and sperm maturation.<sup>15</sup> Subclinical genital tract inflammation can lead to impairment of spermatogenesis by altering cytokine levels, which, in turn, impair Sertoli cell function.<sup>11</sup> Furthermore, polymorphonuclear neutrophils, the predominant type of leukocytes in semen, generate reactive oxygen species (ROS) that can impair sperm motility.<sup>12,13</sup> A significant correlation is detected between leukocytospermia and defects in the tail function of sperm.<sup>11</sup> In addition, teratoasthenozoospermia and necrozoospermia are more common in leukocytospermic men than in normospermic men.<sup>13</sup> However, a meta-analysis which evaluated the impact of leukocytospermia in men attending a fertility clinic found no association between the condition and reduced fertility after ART and with altered semen quality.<sup>16</sup>

It has been demonstrated that leukocytospermia hinders the fertilization potential of spermatozoa by interfering with the acrosome reaction and the fusion of sperm and egg.<sup>11,17,18</sup> Due to this, the presence of WBCs in seminal plasma is considered a significant prognostic factor for failed in vitro fertilization and embryo transfer (IVF-ET).<sup>19</sup> Leukocytes in seminal fluid produce high concentrations of ROS and interferon- $\gamma$ , which can inhibit sperm function and decrease the rate of IVF.<sup>14</sup> Moreover, high concentrations of polyunsaturated fatty acids (PUFAs) in the plasma membrane of sperm increases their vulnerability to oxidative stress.<sup>18,20</sup> Lipid peroxidation of these membranes may result from sperm mitochondrial dysfunction as they make a significant contribution to the oxidative stress of the spermatozoa environment.<sup>21,22</sup> Furthermore, sperm DNA fragmentation has been implicated as an important factor in male infertility; however, the impact of leukocytes on sperm DNA fragmentation has been debated. Some studies have found leukocytes to induce DNA fragmentation,<sup>23</sup> while others have found that DNA damage is not related to different leukocyte subpopulations in the ejaculate.<sup>24</sup> Another possible mechanism of sperm function impairment by leukocytes is ROS-induced cross-damage, which may occur when sperm and

leukocytes co-migrate from the seminiferous tubules to the epididymis.<sup>11</sup>

### Methods of assessing leukocytospermia

There are different methods for assessing leukocytes in semen, including identification of round cells, immunohistochemical staining using monoclonal antibodies, the Endtz test, the peroxidase test, and flow cytometry.<sup>5,6</sup> Leukocytes are difficult to distinguish visually from immature germ cells in semen specimens which have been prepared as wet mounts and examined by manual microscopy. Therefore, confirmatory tests are recommended. The American Urological Association (AUA) and the American Society for Reproductive Medicine (ASRM) recommend wet mount microscopy confirmed with immunohistochemistry for diagnosis of leukocytes in a semen sample. In contrast, the European Association of Urology (EAU) recommends wet mount microscopy confirmed with peroxidase positive staining.<sup>4</sup> The WHO also recommends the peroxidase test, although this test distinguishes only granulocytes and no other WBC types.<sup>3</sup> The gold standard for assessment of WBCs in semen is immunohistochemical staining using monoclonal antibodies against specific WBC subpopulations, but this method is expensive, time-consuming, and not standardized.<sup>25</sup> Monoclonal antibodies targeted against CD45 are used as a pan-leukocytic marker in both immunohistochemical staining and flow cytometry.<sup>26</sup> However, reflexive semen cultures have been shown to be both cost-effective and useful in detecting associated bacterial infections in leukocytospermic patients, such as chronic prostatitis and genital tract infection. Reflexive semen culture in combination with the peroxidase method has been found to be of greater clinical use than the peroxidase method by itself, and reflexive semen culture has been suggested for all samples with elevated WBC count.<sup>27</sup> Moreover, semen culture could also have additional value, as a study of infertile men with asymptomatic leukocytospermia suggested that a lack of correlation between sperm morphology, motility, and concentration and positive semen cultures could indicate early or subclinical infection.<sup>28</sup> Urethral culture after digital prostatic massage is another, even more reliable way to detect microorganisms, leading to bacterial infections associated with

leukocytospermia, but these tests are expensive, disliked by patients, and time-consuming.<sup>29,30</sup>

### Treatment for leukocytospermia

There is no clear agreement on the treatment for leukocytospermia.<sup>5</sup> The AUA and ASRM provide no guidelines for treating leukocytospermia, while the Canadian Urological Association (CUA) believes that there is no indicated treatment for leukocytospermia, but antimicrobial therapy can be considered in an infertility setting. According to EAU guidelines, antibiotics may improve the overall quality of spermatozoa, but there is no evidence of increased pregnancy rates after antibiotic treatment of the male partner.<sup>4</sup>

A systematic review of treatments for leukocytospermia found that antibiotics might improve sperm parameters, the rate of resolution of leukocytospermia, the bacteriological cure rate, and even the pregnancy rate, although reports were conflicting.<sup>5</sup> In vitro studies have shown that antioxidants may also have clinical benefit for sperm function. However, the data were insufficient to conclude whether antibiotics and antioxidants for the treatment of infertile men with leukocytospermia were effective or not.<sup>5</sup>

Brunner and colleagues<sup>4</sup> performed a systematic review of the literature to compare and contrast available international guidelines and recommendations for the evaluation and treatment of leukocytospermia. The review found no clear consensus, with some guidelines describing a lack of correlation between leukocytospermia and infertility, while other guidelines described treatments that improved sperm quality, as evidenced by several outcomes including improved pregnancy rates. These guidelines suggested various treatments, including antibiotics, anti-inflammatory medications, and ejaculation at least once a month.<sup>4</sup> Caution has been advised in the use of antibiotics, as animal studies have found that antibiotics can arrest spermatogenesis and disturb other semen parameters.<sup>31</sup> Thus, care should be taken to administer the appropriate dose and duration of antibiotic therapy to prevent these complications.

Other agents have been used in the treatment for leukocytospermia. For example, ketotifen, an anti-histamine-like drug, was found to improve sperm

motility and morphology in men with leukocytospermia and unexplained infertility.<sup>32</sup> Antioxidants have been used to reduce the production of ROS by seminal leukocytes and improve sperm quality.<sup>33–35</sup> Nonsteroidal anti-inflammatory drugs (NSAIDs) were found to recover sperm count, motility, and morphology in asthenoteratozoospermic men with leukocytospermia.<sup>13,36</sup> Collectively, these studies reflect the continuing controversy regarding the evaluation and treatment of leukocytospermia.

### Laboratory methods of separating WBCs in semen samples

Based on studies showing the negative impact of leukocytospermia on IVF and intracytoplasmic sperm injection (ICSI) outcomes,<sup>4</sup> some laboratory protocols may include removal of WBCs from semen prior to freezing sperm or using sperm in ART procedures. There are a variety of methods for removing WBCs from semen. The oldest method is swim-up from a washed pellet.<sup>37</sup> The benefit of this technique is that it results in a specimen with a very high percentage of motile sperm (90%), an increased percentage of morphologically normal sperm and a significantly decreased number of nonsperm cells such as WBCs. The disadvantages of this technique are that it results in a decreased percentage of normally chromatin-condensed sperm and increased sperm damage by ROS in patients with elevated ROS in the ejaculate or with genital tract inflammation. Because of these disadvantages, the conventional swim-up technique may not be optimal for removing WBCs from leukocytospermic specimens.<sup>37,38</sup>

Glass wool filtration has also been used to separate WBCs from semen. In this method, motile sperm cells are separated from nonsperm cells, including WBCs, by means of densely packed glass wool fibers.<sup>39</sup> The advantages of this method include isolation of sperm from ejaculates with very low sperm concentrations and significant reduction of leukocytes and ROS. The disadvantages are that it is more expensive than the swim-up method, the filtrate is not clean, and remnants of debris are still present.<sup>37</sup>

Mechanical filtration using physical filters such as micropore filters can also be used with leukocytospermic samples to separate sperm cells, but the filters readily clog due to the high number of

WBCs and from various possible tissues in the sample.<sup>40</sup>

Density gradient centrifugation is another method of separating WBCs from semen. It consists of filtering sperm cells by centrifugal force through either one or multiple layers of increasingly concentrated silane-coated silica particles. In this method, highly motile sperm cells move actively in the direction of the sedimentation gradient, and thus highly motile sperm cells are enriched in the soft pellet at the bottom.<sup>37</sup> The advantages of this technique include isolation of sperm from ejaculates with very low sperm concentrations, significant reduction of WBCs, and significant reduction of ROS. The disadvantages of this technique are that it is more time-consuming and expensive than the swim-up technique.<sup>37</sup>

Microfluidics has the potential to separate sperm cells from other cells/cellular debris. This method uses inertial microfluidic technology to separate sperm cells from WBCs by directing the flow of these two cell types into different channels within a spiral chamber. The method does not require labeling cells or applying external forces. Using this approach, it is possible to recover not only motile sperm but also viable less-motile and non-motile sperm cells while at the same time reducing the concentration of WBCs.<sup>41</sup> Ultimately, the choice of method for removing WBCs from semen will depend upon the resources of the laboratory and the overall characteristics of the semen specimen.

### Hemospermia

Hemospermia (also termed hematospermia or bloody ejaculate), is defined as the presence of blood in the ejaculate.<sup>7,8</sup> Although the common age range of men afflicted with hematospermia is between 30 and 40 years (mean, 37 years), it can be seen in men over 40 years. The prevalence of hematospermia is about 1.0–1.5% of all urological referrals; however, the exact incidence is unclear.<sup>8,42,43</sup> It may occur as a single episode or persist chronically<sup>44</sup> with an average duration of 1–24 months.<sup>42</sup> Although a variety of pathophysiological mechanisms are reported for hematospermia, approximately 30–70% of cases are idiopathic.

Disorders of the testis, accessory glands or their ducts, bladder, or urethra can lead to

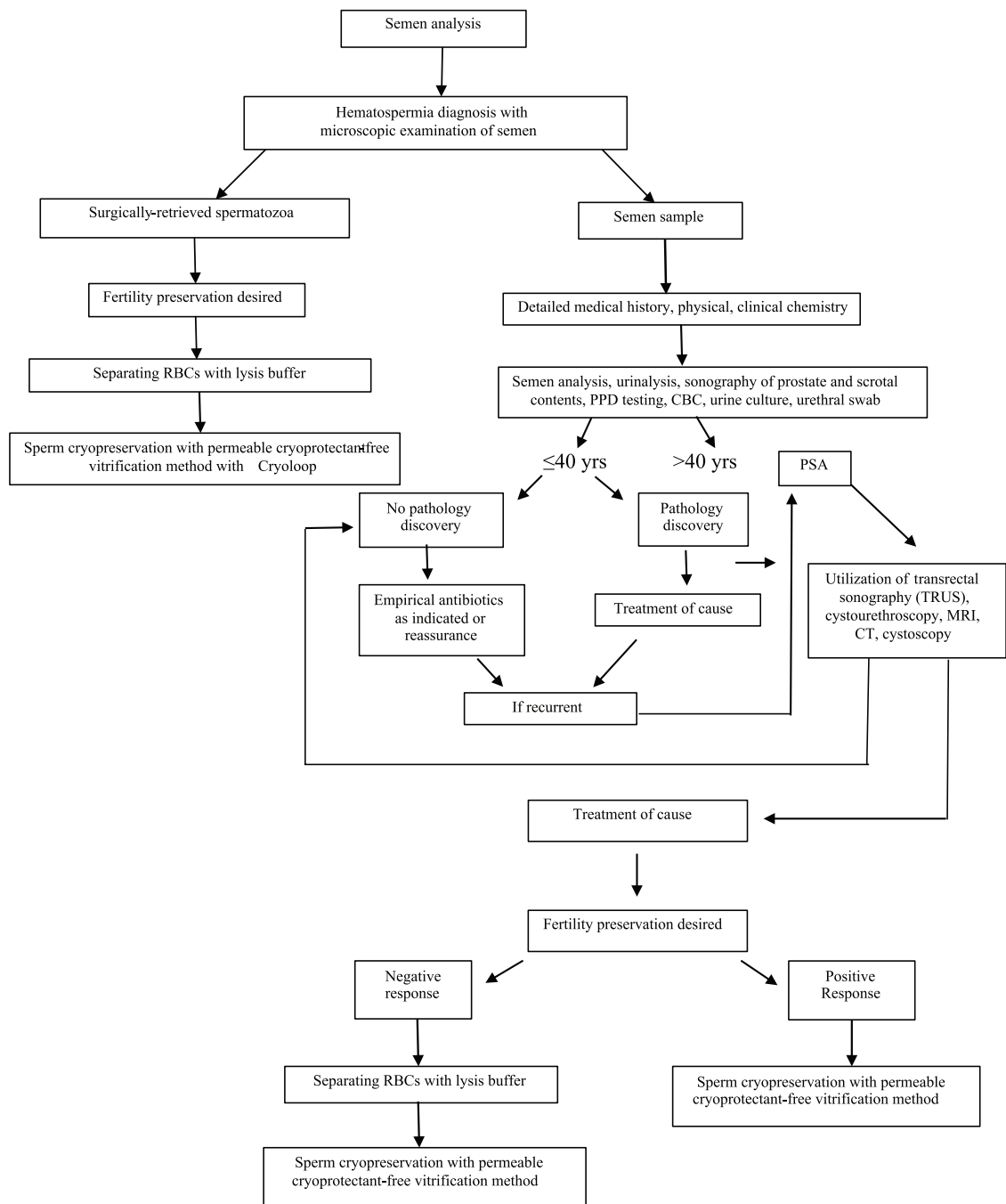
hematospermia.<sup>7</sup> Such disorders may include inflammation, infections, mechanical or chemical agents, ductal obstruction and cysts, tumors and vascular abnormalities, systemic factors, lithiasis, deviant sexual practices, or excessive sexual intercourse/masturbation. Iatrogenic factors are the most common etiologies of hematospermia caused by prostate biopsy, radiation therapy, brachytherapy of prostate cancer, and other urological interventions.<sup>8,42,44,45</sup> Also, surgical sperm retrieval from the testis or epididymis of infertile men commonly results in a specimen contaminated with numerous RBCs.<sup>46</sup> An overview of clinical and laboratory management of hematospermia can be seen in Figure 2.

### Hematospermia and infertility

Hematospermia may clinically present with infertility.<sup>45,47</sup> A 2015 study conducted by a large andrology department on a select group of patients found erythrocytes in the ejaculate of 13.8% of the patients, whose semen had been analyzed generally because of infertility.<sup>9</sup> Toxic substances of RBCs may affect the fertilization potential of spermatozoa, particularly if these samples are to be cryopreserved.<sup>46</sup> In a canine model, blood added to semen (4% v/v) prior to cryopreservation resulted in impaired sperm motility, impaired sperm membrane integrity, and impaired acrosomal status in the thawed specimens. The detrimental effects were attributed in part to the high amount of hemoglobin originating from RBC hemolysis observed after the process of freezing and thawing.<sup>46</sup>

### Diagnostic methods of hematospermia

The initial steps in diagnosing hematospermia include a detailed medical history, physical examination, and clinical chemistry.<sup>42</sup> Sometimes blood is visible macroscopically, but it may also be detected microscopically by semen analysis. The color of ejaculate may differ depending on how much time has passed since the bleeding event. Semen may range in color from light red, if fresh blood is present, to dark brown or black in cases of old bleeding.<sup>9</sup> Diagnosis may be aided by imaging techniques, such as transrectal ultrasonography (TRUS), computerized tomography (CT), Doppler sonographic examination of the testicle and abdomen, magnetic resonance imaging (MRI), and cystoscopy.<sup>8,42</sup>



**Figure 2.** Algorithm for the management of semen samples obtained from hematospermic infertile males.

**Treatment for hematospermia**

Treatment for hematospermia depends on the underlying cause. Management generally depends on the quality and duration of symptoms, age of patient, and the accompanying symptoms.<sup>48</sup> It should be noted that a majority of instances have

no serious underlying disease and are self-resolving.<sup>49,50</sup> It is also common that hematospermia may be related to a recent procedure, such as prostate biopsy, and symptoms are expected to resolve. Patients who are under 40 years of age with an instance of hematospermia should be evaluated

with thorough history-taking, physical examination (prostate and external genitals), urinalysis, sonography of prostate and scrotal contents, purified protein derivate (PPD) testing, and routine chemistry [comprehensive metabolic panel (CMP), complete blood count (CBC), and coagulation studies].<sup>9,51</sup> If workup identifies a specific organism, antibiotic or antiparasitic medication is indicated. However, if infection is still suspected and no organism is identified, empiric antibiotics may be indicated after weighing risks and benefits.<sup>52</sup> Many clinicians offer empiric antibiotics on the basis of presumptive prostatitis due to the rationale that it is relatively common, but the use of this method is controversial, and resolution of symptoms may be attributable to spontaneous resolution. However, if no specific etiology is identified from the diagnostic workup and infection is not suspected, patients should be reassured, and resolution should be expected.

For patients who are older than 40 years of age or patients of any age with recurrent episodes of hematospermia, a more extensive workup is indicated. A more robust urological investigation may include prostate-specific antigen (PSA) levels, TRUS, and cystourethroscopy.<sup>9,53,54</sup> Persistent hematospermia can be an early sign of prostate cancer; thus, PSA should be monitored in older patients or in those with a family history of prostate cancer, and regular follow-up should be scheduled.<sup>51</sup>

A recent prospective placebo-controlled study evaluated the use of finasteride as a treatment for refractory hematospermia. The rationale behind exploring finasteride as a treatment option was due to its role in altering angiogenic factors such as vascular endothelial growth factor (VEGF). Finasteride has been shown to decrease VEGF levels and alter suburethral prostatic microvessel density which could affect their propensity to bleed.<sup>55,56</sup> Results of this study showed that after treatment for 3 months, 67% of patients treated with finasteride had resolution of their hematospermia symptoms when compared with only 25% of resolution in the placebo group.<sup>57</sup> However, the results of this study are preliminary.

Another novel technique is the use of a Holmium laser in the treatment for ejaculatory duct and seminal vesicle diseases causing hematospermia. Results showed that this method represents a

minimally invasive approach to treat calculi in the ejaculatory ducts or seminal vesicles with a resolution rate of 87%.<sup>58</sup>

### Laboratory methods of separating RBCs from semen samples

Based on findings showing the negative impact of hematospermia on sperm parameters,<sup>46</sup> some laboratory protocols include removal of RBCs from semen prior to freezing sperm or using sperm in assisted conception procedures. Different methods have been used to remove RBCs from semen. One study investigated the effect of RBC separation on the outcome of dog semen cryopreservation. The separation methods included swim-up and gradient centrifugation. The quality of post-thaw spermatozoa after gradient centrifugation was higher compared with the swim-up method.<sup>59</sup>

In a canine model, various density gradient media were compared for their ability to separate motile sperm from cellular contaminants. A perfect medium should yield a high number of motile sperm with low contamination by immotile sperm and RBCs.<sup>60</sup> Four commercially available density gradient media were compared: ISolate, Percoll, PureCeption, and PureSperm 100. The results showed that all four media were effective in separating sperm from RBCs; however, PureCeption more efficiently separated the motile sperm from RBCs.<sup>60</sup> Although this study included Percoll, it should be noted that Percoll has been removed from clinical application in human reproduction since 1996, due to the risk of endotoxin contamination.<sup>61</sup>

Recently, microfluidics has been employed for a variety of applications in ART such as gamete handling.<sup>62</sup> Son and colleagues demonstrated the capacity of a microfluidic spiral device to separate sperm from RBCs and other debris. The advantage of this technique is that sperm separation takes a few minutes. In addition, it is independent of sperm motility, and the separated spermatozoa are label-free, which allows the use of sperm retrieval from testicular sperm extraction (TESE)/micro-TESE specimens for the ARTs.<sup>63</sup>

The use of RBC lysis buffer has been suggested to decrease the amount of time it takes to find sperm within testis biopsy specimens. These specimens

have a notoriously high RBC to sperm cell ratio, making it difficult and time-consuming to find the few sperm cells among the many RBCs. Time is a critical factor when sperm are to be used in an IVF or ICSI procedure. A study by Nagy and colleagues found that treatment with RBC lysis buffer decreased the time required to find sperm in the pellets of testicular biopsies, and the use of RBC lysis buffer did not affect the rates of fertilization or embryo development. In addition, the rates of transferred or frozen embryos were similar to those in the conventional method to recover sperm from testis biopsy specimens.<sup>64</sup>

### **Sperm cryopreservation methods for samples with RBCs and WBCs**

Sperm cryopreservation is often recommended to preserve fertility in men who have medical conditions that may impair future fertility. However, ROS production in samples cryopreserved while containing WBCs and RBCs may negatively impact the quality of the specimens.<sup>2,46</sup> No clear clinical strategies exist for how to manage the cryopreservation of samples with leukocytospermia or hematospermia. Therefore, using appropriate strategies to eliminate WBCs and RBCs and minimize ROS production in semen samples can increase the efficiency of sperm cryopreservation. Following WBC and RBS elimination, slow freezing, rapid freezing, and ultra-rapid freezing are the main cryopreservation methods used for these patients.<sup>65</sup>

Slow freezing is a manual or automated method in which sperm cryopreservation is performed by adding cryoprotectant to the semen specimen and then gradually lowering the temperature of the specimen over a 1- to 2-h period. Without the addition of a cryoprotectant, high concentrations of electrolytes inside the sperm cells can lead to the formation of ice crystals and physical-chemical injury. Control of the cooling rate is an important factor to minimize osmotic injury during the process of slow freezing.<sup>2,65,66</sup> However, even with these precautions (addition of cryoprotectant and a slow rate of cooling), the slow freezing method can lead to changes in the structure and function of sperm. Furthermore, this method is laborious and time-consuming.<sup>1,67-69</sup>

In the rapid freezing method, a cryoprotectant agent is mixed with the semen sample, which is

then placed into a cryovial or cryo-straw and exposed to liquid nitrogen vapor before being plunged into liquid nitrogen.<sup>66</sup> One benefit of the rapid freezing method is the prevention of ice crystal formation.<sup>70</sup> As classical vitrification requires a high percentage of permeable cryoprotectants leading to possible lethal osmotic effects and chemical changes, it appears that this approach is not suitable for sperm vitrification.<sup>71</sup> In kinetic vitrification as an ultra-rapid method, spermatozoa are frozen without the permeable cryoprotectants. In this method, the sperm suspension is directly immersed into liquid nitrogen in an ultra-rapid cooling procedure.<sup>71</sup>

The above-mentioned methods for sperm cryopreservation are not perfect methods to cryopreserve low numbers of sperm retrieved from the epididymis and testicles. Therefore, newer techniques using various “carriers” to gather small quantities of sperm have been used for cryopreservation of testicular and epididymal spermatozoa. The ideal carrier would allow the freezing of multiple tiny aliquots of sperm or even individually isolated sperm in small numbers (i.e. 5–10 sperm per carrier), thus conserving the very small supply of motile sperm in these specimens. This would prevent repeated freezing/thawing of the original specimen and allow multiple IVF attempts.

AbdelHafez and colleagues<sup>72</sup> reviewed various carriers. Biological carriers have been used for this purpose, such as an empty zona pellucida. In this method, individually selected spermatozoa are stored inside an animal or human zona pellucida which has been evacuated of its cytoplasmic contents using micromanipulation tools. The zonae are then frozen in liquid nitrogen. Another biological carrier that has been used to cryopreserve small quantities of sperm are spheres of *Volvox Globator* algae. Colonies of this alga consist of 1500–20,000 cells held tightly in a sphere-like structure. Spheres are injected with motile sperm using an ICSI needle. The spheres, which may also contain a cryoprotectant, are then frozen in straws. In addition, nonbiological carriers have been used to store small quantities of sperm, including mini straws, microdroplets, cryoloops, ICSI pipettes, calcium alginate beads, and agarose gel microspheres. The review concluded that there was insufficient evidence to recommend any particular carrier method.<sup>72</sup>



Several studies have compared various methods of sperm cryopreservation. According to these studies, slow freezing provides higher post-thaw sperm quality than vitrification.<sup>73,74</sup> Hammadeh and colleagues<sup>75</sup> have reported that higher chromatin damage occurs in the rapid freezing method when compared with the slow freezing method, while Vutyavanich and colleagues<sup>76</sup> showed that motility and viability of sperm in the rapid freezing method were higher than those in the slow freezing method. Sperm cryopreservation decreased sperm DNA integrity, morphology, and motility following both the ultra-rapid freezing and the slow freezing methods. Nevertheless, sperm motility decreased more by ultra-rapid freezing.<sup>77</sup> Births of healthy babies have been reported following ICSI using the permeable cryoprotectant-free sperm vitrification.<sup>71</sup> Findings from a recent study have shown that the permeable cryoprotectant-free vitrification can present significantly higher sperm quality parameters than slow freezing.<sup>67</sup> In addition, DNA fragmentation and acrosome damage are decreased in vitrified samples.<sup>67</sup> With kinetic vitrification, the additional step of centrifugation is reduced using sperm-free seminal plasma for cryopreservation. Thus, it has been suggested that kinetic vitrification is the most appropriate option for use in clinical trials.<sup>78,79</sup>

### Conclusion

The presence of WBCs and RBCs can significantly affect semen quality and fertility. ROS production is the most prominent issue and is further exacerbated when these specimens containing WBCs or RBCs are cryopreserved. Proper diagnostic methods should be utilized, and underlying etiologies should be ruled out using more extensive urological workups in recurrent and high-risk cases. Treatment options for these conditions have proven controversial as studies have demonstrated conflicting results. Currently, no clear clinical strategies exist for how to manage the cryopreservation of samples with leukocytospermia or hematospermia. However, appropriate strategies to eliminate WBCs and RBCs in semen samples can improve outcomes for cryopreservation and ART.

### Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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