Microbiological investigation in male infertility: a practical overview

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The roles of inflammation and/or infection of the male accessory sex glands are very important for the potential effects that these conditions may have on male fertility. The clinical andrologist should be aware of the pathophysiological role of the main determinants of sperm damage when these conditions occur, in particular, seminal leukocytes, oxidative stress and cytokines. In addition, it is important to have a good knowledge of the methodologies to be used in clinical practice. This article summarizes the methods used to look for and to identify the micro-organisms responsible for male urogenital tract infections. These include sperm culture, urine culture, urethral swabbing, the Meares–Stamey test and balanopreputial swabbing. Finally, we discuss the role of human papilloma virus infection in male infertility.

Introduction

Since the ejaculate is a mixture of secretions derived from the urogenital tract and the male accessory glands, seminal culture identifies the presence of germs in any section of the seminal tract (De Francesco et al., 2011). In clinical practice, cultural examination of the ejaculate can result in false positive or negative findings either due to germs present on the glans, prepuce and hands of the patient or because of saprophytic bacteria in the anterior urethra (Askienazy-Elbhar, 2005).

In the pre-analytical phase, to minimize the risk of bacterial contamination (false positives), it is appropriate to give accurate information on improved hygiene and collection procedures to the patient. False negatives may result from the interference of pathogenic bacteria in the culture medium due to bactericide production by Gram-positive bacteria. To prevent false negatives, it is necessary to dilute the seminal fluid with saline before plating it onto agar media (Askienazy-Elbhar, 2005; De Francesco et al., 2011; Boitrelle et al., 2012).

In the analytical phase, microbiological investigations should consider the germs under investigation as belonging to one of the following three main categories: ‘certain pathogens’, ‘likely pathogens’ (occasional) and ‘potential pathogens’ (which have a controversial role). Several factors contribute to this categorization, including lifestyle, sexual promiscuity, poor hygiene and intestinal habits. In addition, medical and clinical (objective and ultrasonographic) factors associated with complicated urinary tract infection (UTI) and male accessory gland infection (MAGI) as well as abnormal conventional sperm parameters (concentration, motility and morphology) and abnormal unconventional sperm parameters (mitochondrial function and/or DNA integrity) also contribute to this categorization (Askienazy-Elbhar, 2005; De Francesco et al., 2011; Boitrelle et al., 2012).

UTI is an infection of a part of the urinary tract. When UTI affects the lower urinary tract, it is known as a simple cystitis and when it affects the upper urinary tract it is known as pyelonephritis. Symptoms from a lower UTI include painful urination and either frequent urination or the urge to urinate or both, while those of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. In the elderly and the very young, symptoms may be vague or non-specific. The main aetiological agent of both types is Escherichia coli; other bacteria, viruses or fungi may rarely be the cause (Rusz et al., 2012).

MAGI results from the canalicular spreading of microorganisms via the urethra, prostate gland, seminal vesicles,
deferent duct, epididymis and testis. Haematogenous infections are rare. The main infectious agents are *Neisseria gonorrhoeae, Chlamydia trachomatis* and enterobacteria (the latter with a lower frequency than the other infectious agents). Characteristic symptoms of MAGI are leukocytospermia and enhanced concentrations of cytokines and reactive oxygen species (ROS) (La Vignera et al., 2011).

Some Gram-negative bacteria (*Enterobacteriaceae* such as *E. coli, Klebsiella* species, *Proteus, Serratia, Pseudomonas* species, etc.) and aetiological agents of sexually transmitted diseases (*C. trachomatis, Ureaplasma urealyticum, Treponema pallidum, N. gonorrhoeae*, etc.) are recognized as ‘certain pathogens’ of the prostate (category II, National Institutes of Health classification) (Krieger et al., 1999; Nickel et al., 1999) because they show a close association with positive history (past and/or recurrent UTIs, sexually transmitted diseases, urogenital congenital anomalies) and/or positive physical examination for urogenital abnormalities (phimosis, hypospadias, cryptorchidism). Instead, some micro-organisms of the prostate, which are occasionally detectable in the urogenital tract, are considered by some authors to be ‘non-pathogenic’, ‘likely pathogens’, ‘occasional pathogens’ (Gram-positive germs, such as *Enterococcus* spp., *Staphylococcus aureus*, obligate anaerobes) or ‘possible pathogens’ (coagulase-negative germs, such as *Staphylococcus haemolyticus, Staphylococcus epidermidis, mycoplasmas*) (Krieger et al., 1999; Nickel et al., 1999).

The main difficulties in the interpretation of laboratory microbiological data (seminal and/or urine culture) is the presence of contaminating microbiota, commensal flora of the anterior urethra, bacteriostatic or bactericidal substances in the prostatic secretions and previous antibiotic treatments. This is why the diagnosis of bacterial prostatitis (category II, National Institutes of Health classification; World Health Organization (WHO) criteria for MAGI) (Krieger et al., 1999; Rowe et al., 1993) must be confirmed either by quantitative seminal culture (c.f.u. >10⁵ for certain pathogens or >10⁴ for non-pathogenic agents, after dilution of the seminal plasma with saline) (Comhaire et al., 1980) or by cultures obtained from urinary stream fractionation using the four-glass test (Meares & Stamey, 1968) and/or the two-glass test (Nickel, 1997).

Finally, chronic and prolonged exposure to one or several microbial noxae on one or more male accessory sex glands causes secretory dysfunction and has a negative impact on sperm bio-functional competence; however, before these effects occur, the site of the infective/inflammatory process in the gland shows multiple echo-morphostructural alterations. With respect to this, we evaluated infertile men with MAGI and elevated bacteriospermia (>10⁶ c.f.u. ml⁻¹) by testicular and prostate-vesicular transrectal ultrasound and we found a large number of ultrasonographic abnormalities that involved more than one male accessory gland (Vicari, 1999; Vicari et al., 2006). Infertile patients with MAGI have a heightened inflammatory response (in terms of leukocytospermia and ROS overproduction) and a parallel alteration of sperm parameters that are directly related to the anatomical extension of MAGI (prostatitis ≤prostate-vesiculitis ≤prostate-vesiculo-epididymitis) (Vicari, 1999). In view of these findings, the quantitatively significant threshold value for the seminal culture that best correlates with other clinical and laboratory findings of MAGI is >10⁵ c.f.u. ml⁻¹.

The role played by the micro-organisms responsible for male urogenital infections and their impact on conventional and unconventional sperm parameters, as well as their hypothetical pathogenic mechanisms, have recently been reviewed (La Vignera et al., 2011).

A strong association between inflammation of the male reproductive system and infertility has been reported (Comhaire et al., 1999; La Vignera et al., 2011). In particular, semen quality is altered by the inflammatory process through the impaired secretory capacity of the accessory glands, anatomical obstruction, the presence of an unsuitable micro-environment and/or spermatogenesis dysregulation (Comhaire et al., 1999; La Vignera et al., 2011). Several analyses of genito-urinary tract inflammation suggest that a redox imbalance in the semen is a particularly important mediator of the cause/effect relationship between semen infection and spermatozoon functional deficiency (Aitken et al., 1989; Aydemir et al., 2008). It is believed that ROS overproduction associated with inflammatory reactions is caused by pathological bacterial strains that colonize or infect the reproductive tract (Comhaire et al., 1999; La Vignera et al., 2011).

In a simplistic model, the kinetics of the urogenital tract inflammatory process may be envisaged in several different phases (Fraczek & Kupriz, 2007). The presence of bacteria and/or leukocytes in semen represents the initial element. Subsequently, ROS overproduction causes an oxidative imbalance and the accumulation of leukocytes is associated with the initiation of phagocytosis. The activation of specific receptors and signal transduction pathways then occurs, generating biologically active substances such as pro-inflammatory cytokines. These substances then modulate pro- and anti-oxidative system activation and promote a burst of ROS. Another phase is represented by spermatozoon peroxidative damage. Finally, remnants of the oxidative stress response may persist in the semen for a long period of time after the infectious agent has been eradicated, further damaging spermatozoa.

### The importance of identifying MAGI during infertility diagnosis

MAGI has a negative impact on reproductive function. According to the WHO (Rowe et al., 1993), MAGI is diagnosed when abnormal sperm parameters are found associated with at least one A factor plus one B factor, one A factor plus one C factor, one B factor plus one C factor, or two C factors (Table 1). In the management of male infertility, the andrologist should consider that although...
bacterial prostatitis (Meares & Stamey, 1968; Nickel et al., 2006). In our opinion, an ultrasound scan of the prostate may improve the reliability of this test, since the operator may more accurately direct the massage to the prostate lobe that shows signs of inflammation on ultrasound. In fact, ultrasound is an excellent tool to distinguish unilateral from bilateral forms of prostatitis (Vicari et al., 2006).

Semen leukocyte concentration

Semen leukocytes represent an important problem in clinical practice. In fact, leukocytes, predominantly polymorphonuclear leukocytes (neutrophils), are present in human ejaculates. Differentiation between neutrophils and spermatocytes or spermatids is based on the size and shape of the stained cell nuclei. Neutrophils are easily confused with polymnucleated spermatids, but they become blue when coloured with the May–Grunwald staining, whereas spermatids become pink. The size of the nucleus can further be used to help distinguish between monocytes, lymphocytes (approximately 7 μm) and macrophages (approximately 15 μm), even if their functional changes cause an abnormal level of variability in this parameter. The detection of leukocytes in human semen is performed by the use of cytochemistry to identify a peroxidase enzyme that is present in granulocytes. This technique has, however, two major disadvantages: it does not detect activated granulocytes that have released their granules and it does not detect other types of leukocytes that do not express peroxidase, such as lymphocytes, macrophages and monocytes.

Finally, oxidative stress and cytokine release are regarded as the main mechanisms through which the inflammation of the male genital tract negatively affects sperm parameters.

**Evidence for the effects of oxidative stress**

The increased ROS production and/or the decrement of scavenger activity may cause spermatic abnormalities. These abnormalities include decreased sperm motility, acrosine activity and sperm-oocyte fusion capability (for a review, see Lanzafame et al., 2009). Accordingly, a sperm-oocyte penetration rate of less than 25% is associated with increased ROS production in a number of oligozoospermic patients (Aitken et al., 1989). ROS-induced inhibition of sperm motility correlates negatively with malondialdehyde (MDA) seminal plasma levels (Saraniya et al., 2008), whereas low levels of MDA are associated with an increased oxidative stress.

**Table 1. Male accessory gland infection: WHO diagnostic criteria (Rowe et al., 1993)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>History: positive for urinary infection, epididymitis and/or sexually transmitted disease</td>
</tr>
<tr>
<td>B</td>
<td>Prostatic fluid: abnormal prostate fluid expression and/or abnormal urine after prostatic massage</td>
</tr>
<tr>
<td>C</td>
<td>Ejaculate signs: leukocyte &gt;1 million ml⁻¹, culture with significant growth of pathogenic bacteria, abnormal appearance, increased viscosity, increased pH and/or abnormal biochemistry of the seminal plasma</td>
</tr>
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From a practical point of view, three different aspects of inflammation should be evaluated: biochemistry, microbiology and leukocyte concentration in the seminal fluid.

**Semen biochemistry**

Secretions from accessory glands can be measured to assess their function. In particular, citric acid, zinc, γ-glutamyl transpeptidase and acid phosphatase may be used to evaluate prostatic function. Fructose and prostaglandins may be used to evaluate the secretory capacity of the seminal vesicles and free L-carnitine, glycerophosphocholine and neutral α-glucosidase may be used to evaluate the function of the epididymis. There are two isoforms of α-glucosidase present in the seminal plasma; the neutral form (the major constituent) originates from the epididymis and the acid form originates from the prostate.

**Semen microbiology**

Theoretically, the aetiology of prostatitis may involve any species of bacteria (aerobic or anaerobic), yeast or other cryptic micro-organisms (Krieger et al., 1999). The appropriate microbiological approaches therefore include the identification of a low number of bacteria in expressed prostatic secretions or post massage urine, which can be pathognomonic of chronic bacterial prostatitis (Budia et al., 2006). One of the major microbiological problems in prostatitis bacteriology is in determining which bacteria can be considered true pathogens. There are two different approaches to this problem: (a) consider as pathogens only those bacteria present in expressed prostatic secretions or bacteria known to be a cause of recurrent UTI or (b) consider as pathogens those bacteria present in a single biological sample (urethral swab and/or urine or semen culture). The Meares and Stamey test is currently considered the most important test for the diagnosis of bacterial prostatitis (Meares & Stamey, 1968; Nickel et al., 2006).
pregnancy rate (Suleiman et al., 1996). An increased oxidative stress causes seminal plasma hyperviscosity in infertile patients (Aydemir et al., 2008) and damages sperm chromatin/DNA integrity. Indeed, increased ROS production results in: (a) a greater number of normal spermatozoa with DNA fragmentation (Aitken et al., 1998) and (b) a greater frequency of single- and double-strand DNA breaks (Barroso et al., 2000); and (c) increased DNA–protein cross-linking (Twigg et al., 1998). DNA damage in spermatozoa may lead to an increased risk of miscarriage and chromosomal abnormalities (Griveau & Lannou, 1997).

**Effects of pro-inflammatory cytokines**

Cytokines have pleiotropic and redundant effects during the inflammatory response. Hence, they disrupt male accessory gland function with different mechanisms.

**Interleukin 1**

Seminal plasma interleukin (IL)-1 (IL-1) concentrations are higher in patients with infertility than in normal controls. However, the levels of IL-1 are similar in patients with similar sperm motility or morphology (Dousset et al., 1997). IL-1 does not have any effect on the acrosome reaction (Dimitrov & Petrovska, 1996) or on MDA production (Fraczek et al., 2008) in normal spermatozoa.

**Interleukin 6**

IL-6 concentration in the seminal plasma is higher in infertile patients than in fertile men and correlates negatively with sperm MDA. This suggests that it is involved in the onset of ROS-mediated lipoperoxidation (Camejo et al., 2001). IL-6 has been reported to decrease sperm motility in vitro; this effect seems to be due to nitric oxide overproduction (Lampiao & du Plessis, 2008). Similar to IL-1, IL-6 inhibits the acrosome reaction in normal spermatozoa, although to a lesser extent than does tumour necrosis factor-alpha (TNFz) (Lampiao & du Plessis, 2009).

**Interleukin 8**

IL-8 has no effect on sperm motility or on acrosome reaction in vitro (Fedder & Ellermann-eriksen, 1995). In contrast, seminal plasma IL-8 correlates negatively with sperm motility and with the number of motile spermatozoa recovered by the swim-up method in infertile patients. The number of semen leukocytes correlates significantly with IL-8 concentration (Eggert-Kruse et al., 2001). Evidence is emerging on IL-8 involvement in inflammation of the prostate, seminal vesicles and epididymis (Lotti & Maggi, 2013). Moreover, an association between IL-8 levels and colour Doppler ultrasound characteristics suggestive of inflammation of the male genital tract has been reported (Lotti et al., 2011). IL-8 is strongly related to leukocytospermia and a tight inverse correlation with ejaculate volume has been shown, suggesting an association with distal male genital tract subobstruction (Lotti & Maggi, 2013).

**Interferon-γ**

Interferon-γ (IFNγ) significantly suppresses sperm motility (Fedder & Ellermann-eriksen, 1995). Although this effect was originally confirmed in experiments using TNFz and IFNγ concomitantly (Estrada et al., 1997), Sikka and colleagues failed to replicate this finding (Sikka et al., 2001). IFNγ increases sperm membrane lipoperoxidation at physiological concentrations; no additional effect was found at higher concentrations, such as those found during infection/inflammation (Martínez et al., 2007). IFNγ has no significant effect on the calcium ionophore-induced acrosome reaction (Fedder & Ellermann-eriksen, 1995), whereas it suppresses spontaneous acrosome reaction rate and acrosine activity (Bian et al., 2007). IFNγ inhibits the Na+/K+-ATPase, Ca2+-ATPase and superoxide dismutase activities and increases nitric oxide production in normal spermatozoa (Bian et al., 2007).

**Macrophage migration inhibitory factor**

During the epididymal transit, mammalian spermatozoa begin to express new proteins involved in motility attainment and sperm fertilizing capability. Macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, is one constituent of the seminal plasma. Northern and Western blot analyses as well as immunohistochemical studies have shown that MIF is expressed all along the epididymis, with a higher level of transcription in the proximal segment. There is a negative correlation between the amount of sperm-associated MIF and sperm motility (Frenette et al., 2005). MIF inhibits motility, increases phosphatidyserine externalization and DNA fragmentation of normal spermatozoa in vitro (Aljabari et al., 2007). The detrimental effect of MIF on sperm motility was confirmed by other authors, but only at high concentrations (Carli et al., 2007).

**Tumour necrosis factor-α**

The data in the literature regarding the effects of TNFz are inconsistent.

Evidence for negative effects on sperm parameters. TNFz levels are higher in patients with bacterial or mycoplasma infections than in men without infection (Gruschwitz et al., 1996). In addition, patients with leukocytospermia (Omu et al., 1999) and/or bacteriospermia (Omu et al., 1999) release greater amounts of this cytokine. In contrast, TNFz inhibits in vitro sperm motility (Hill et al., 1987) and the ability of sperm to fertilize hamster oocytes (Hill et al., 1989). A negative correlation between TNFz and motile spermatozoa was found in patients with bacterial or mycoplasma infection (Gruschwitz et al., 1996). We reported that TNFz inhibits both total and progressive
sperm motility in a concentration- and time-dependent manner (Perdichizzi et al., 2007). This negative effect is related to changes in sperm mitochondrial function (Perdichizzi et al., 2007) and increased nitric oxide production (Lampiao & du Plessis, 2008). TNFα has been reported to increase MDA production at both physiological concentrations and, to a greater extent, at the concentrations observed during infection/inflammation (Martínez et al., 2007). TNFα inhibits spontaneous and induced acrosome reactions in normal spermatozoa (Dimitrov & Petrovska, 1996; Lampiao & du Plessis, 2009). Finally, TNFα causes increased sperm phosphatidylserine externalization and DNA fragmentation (Perdichizzi et al., 2007). A positive correlation between seminal plasma TNFα levels and the percentage of spermatozoa with phosphatidylserine externalization has been reported (Allam et al., 2008).

Evidence for no effect on sperm parameters. Sperm motility (Winckel et al., 1991) and viability (Lewis et al., 1996), hamster oocyte penetration (Winckel et al., 1991) and the acrosome reaction (Fedder & Ellermann-eriksen, 1995) are not affected by TNFα. No relationship between seminal plasma TNFα and sperm parameters was reported in healthy men (Hussenet et al., 1993). TNFα does not correlate with total sperm number (Koçak et al., 2002) and has no effect on MDA production in spermatozoa isolated by swim-up (Fraczek et al., 2008).

Evidence for male accessory gland functional abnormalities after infection

A functional insufficiency of one or more glands can be recognized by measuring seminal plasma concentration of the compounds secreted by these glands. Many of these products are of paramount importance for sperm function. Epididymal secretion products are involved in sperm maturation. Epididymal function can be evaluated by measuring seminal plasma L-carnitine, glycercylphosphorylcholine and/or α-glucosidase concentrations. There is no consensus about the effects of chronic epididymitis on the α-glucosidase level (Mahmoud et al., 1998; Cooper et al., 1990). The seminal vesicles produce fructose, ascorbic acid, ergothioneine, prostaglandins and bicarbonate. These molecules act as reducing agents and prevent sperm agglutination (Okamura et al., 1986). The negative impact of vesiculitis on seminal plasma fructose concentration is controversial (Comhaire et al., 1989; Cooper et al., 1990). The secretory function of the prostate gland may be evaluated by measuring seminal plasma pH, citric acid or γ-glutamyl transpeptidase levels. The seminal plasma concentrations of these factors are usually altered during infection and inflammation (Weidner et al., 1999). However, they are not recommended as markers of prostatitis (Ludwig et al., 2002).

Sperm concentration, as well as seminal plasma α-glucosidase, fructose and zinc concentrations, have been reported to be significantly lower in patients with urogenital infection than in non-infected men. Decreases in the concentrations of these factors are associated with lower seminal volume and higher pH. However, receiver operating characteristic (ROC) analysis showed that none of these parameters was sufficiently accurate to discriminate between infected and non-infected men (Marconi et al., 2009). These findings showed that men with proven urogenital infection have poorer accessory gland function than in non-infected men, suggesting that the epididymal, vesicular and prostate secretory capacity is impaired. However, none of the semen parameters evaluated can be used to reliably diagnose infection.

Description of microbiological methods

The microbiological investigations of the male urogenital tract are often difficult to perform because the sample is not specific to any specific site and the sample may contain more than one type of micro-organism. It is therefore important for the clinician to formulate a diagnostic hypothesis that enables the microbiologist to seek the correct pathogenic micro-organism. The following tests are usually performed: seminal culture, urine culture, urethral swabbing, the Meares–Stamey test (Meares & Stamey, 1968) and balanopreputial swabbing.

Seminal culture

Indications. Sperm culture enables the detection of micro-organisms that are responsible for infection in one or more male accessory sex glands and allows an aetiological diagnosis to be made. Therefore, the cultural examination of the ejaculate is useful in screening for male infertility. The search must be extended to all micro-organisms that may alter sperm function (La Vignera et al., 2011; Leterrier et al., 2011).

Micro-organisms to be searched for. The microbiological investigation of semen must focus on the following micro-organisms:

1. Gram-negative bacteria (Enterobacteriaceae such as E. coli, Klebsiella species, Proteus, Serratia, Pseudomonas species, etc.) recognized as ‘certain pathogens’; micro-organisms considered to be ‘likely, occasional pathogens’ (Gram-positive bacteria, such as Enterococcus species and S. aureus, obligate anaerobes) or ‘possible, controversial pathogens’ (coagulase-negative germs, such as Staphylococcus haemolyticus, Staphylococcus epidermidis and Mycoplasma) for prostate infection;

2. (according to clinical indications and the partner’s clinical history, sperm culture may be extended to the aetiological agents of sexually transmitted diseases (Trichomonas vaginalis and fungi);

3. in patients with previous antibiotic therapy, sperm culture should be extended to germs promoting superinfection (Pseudomonas and fungi).

Sampling methods. In the pre-analytical phase, information must be given to patients regarding the collection procedures (masturbation), the length of sexual abstinence (3–4 days) and abstention from chemo-antibiotic treatment for at least 1 week prior to sample collection. It is essential not to contaminate the sample during or after collection. For this reason, collection must be preceded by accurate hand and external genitalia scrubbing. The patient should urinate prior to sperm collection in a sterile container.

The microbiological search must simultaneously involve the first urine flow and the seminal fluid to better differentiate a UTI from an...
infection of the seminal tract. The first urinary stream also allows for the detection of T. vaginalis and N. gonorrhoeae (after centrifugation), which are rarely isolated from semen due to the presence of substances that inhibit their growth. The sample must reach the laboratory within 1 h from collection. If this is not possible, it should be collected on the premises provided by the laboratory.

**Cultural examination.** Culture focuses on fungi, aerobic organisms, *Gardnerella vaginalis* and mycoplasmas. Some bacteriostatic properties of the seminal plasma are related to the production of bactericides (by Gram-positive species and *N. gonorrhoeae*). Before sowing on the selective specific medium, 1 ml of the semen sample should be diluted (1:10) with sterile saline and centrifuged at 1500 r.p.m. for 15 min. After removing the supernatant, the sediment should be sown using 10 µl calibrated loops. This procedure increases the cultural sensitivity because it concentrates bacteria in the cell pellet and eliminates the seminal plasma that could exert an inhibitory effect on bacterial growth (Vicari et al., 1986). Culture media, conditions and incubation times are summarized in Table 2. To detect *U. urealyticum* and *Mycoplasma hominis*, the culture media used are similar to those described for urethral swabs (see below).

**Identification and sensitivity.** The indications are the same as those described for the urethral swabs. Any aerobic Gram-positive or Gram-negative micro-organisms present in a concentration greater than 10⁶ c.f.u. ml⁻¹ must be identified and tested for antibiotic resistance. If the culture has three or more bacteria, the investigation should be stopped, as this may indicate polymicrobial flora of contamination. For the culture has three or more bacteria, the investigation should be stopped. As this may indicate polymicrobial flora of contamination. (1) If other micro-organisms that are considered ‘certain’ (Gram-negative), antibiotic testing, *M. hominis*, and *Ureaplasma urealyticum* must be placed in a vial containing the transport medium and can be stored at room temperature for 8 h or at 2–8 °C for 36 h. The swab used to detect *C. trachomatis* must be collected and stored following the guidelines depending on the detection method used. Finally, the material obtained from the swab to detect *N. gonorrhoeae* should be sown without delay on a Thayer–Martin agar plate kept at room temperature (or better, pre-warmed to 37 °C) and incubated immediately. Alternatively, the swab may be sent to the laboratory as soon as possible in a specific transport medium without glycerophosphate. In Stuart–Amies medium at room temperature, gonococci survive for 12 h. Once sown, the medium must be incubated at 37 °C in an atmosphere of 5 % CO₂ for 48 h.

**Methods of conservation and transport of the urethral swabs.** Swabs taken to detect fungi or aerobic bacteria must be transported to the laboratory in a specific medium and stored at room temperature for no more than 24 h. The swab used to detect *T. vaginalis* must be maintained in the appropriate transport medium/culture (37 °C) for 15 min and then must be sent promptly to the laboratory. The swab used to detect *Mycoplasma/Ureaplasma* must be placed in a vial containing the transport medium and can be stored at room temperature for 8 h or at 2–8 °C for 36 h. The swab used to detect *C. trachomatis* must be collected and stored following the guidelines depending on the detection method used. Finally, the material obtained from the swab to detect *N. gonorrhoeae* should be sown without delay on a Thayer–Martin agar plate kept at room temperature (or better, pre-warmed to 37 °C) and incubated immediately. Alternatively, the swab may be sent to the laboratory as soon as possible in a specific transport medium without glycerophosphate. In Stuart–Amies medium at room temperature, gonococci survive for 12 h. Once sown, the medium must be incubated at 37 °C in an atmosphere of 5 % CO₂ for 48 h.

**Analytical phase: pathogens to be examined.** When urethritis from sexually transmitted diseases is suspected, the presence of the following pathogens should be evaluated: *C. trachomatis* (observed in 23–35 % of non-gonococcal urethritis), *U. urealyticum*, *M. hominis* and *N. gonorrhoeae*. *T. vaginalis* and fungi are rarely the cause of urethritis; they should be taken into account particularly in cases of persistent symptoms, partner infection, dysuric symptoms and/or post-antibiotic administration balanoposthitis.

If other micro-organisms that are considered ‘certain’ (Gram-negative bacteria, such as *Enterobacteriaceae*) or ‘possible’ (Gram-positive bacteria) are detected, antibiotic treatment must be reviewed.

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**Table 2. Culture media, conditions and incubation times for semen**

Incubation temperature was 37 °C for all cultures.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Culture medium</th>
<th>Incubation conditions</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic bacteria</td>
<td>Blood agar</td>
<td>Aerobiosis</td>
<td>24 h</td>
</tr>
<tr>
<td>Fungi</td>
<td>Sabouraud agar</td>
<td>Aerobiosis</td>
<td>48 h</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>Gardnerella agar</td>
<td>5 % CO₂</td>
<td>48 h</td>
</tr>
<tr>
<td>Others</td>
<td>Chocolate agar</td>
<td>5 % CO₂</td>
<td>48 h</td>
</tr>
</tbody>
</table>
bacteria, such as *Streptococcus faecalis*, *Haemophilus* species, *Streptococcus* species, etc.) pathogens for the urinary tract are detected in the urethral swab, then these should be searched for in the seminal fluid. These acquire real significance only in the presence of a high microbial load associated with other laboratory signs of infection/inflammation (increased polymorphonuclear cells, symptoms of irritation; see diagnostic criteria for MAGI).

**Identification phase.** (Isemberg, 1998; Schiefer, 1998; Murray *et al.*, 2003; Vesic et al., 2007; Orellana *et al.*, 2009.)

For the bacteriological examination, the slide for microscopic examination must be prepared by rotating the swab on the slide and drying the material in the air. The dried glass slide will be inserted into the transport container and can be stored at room temperature until delivery to the laboratory. It is important that it is prepared immediately after collection and not after the swab has been sent to the laboratory. The slide should be subjected to Gram staining and observed at $\times 400$ and $\times 1000$ magnifications. The identification of fungi, leukocytes and microbial species, which are distinct based on their morphology and Gram staining, must be expressed using a semiquantitative system ($+, ++, +++$). The observation of typical Gram-negative intracellular diplococci is strongly indicative for *gonorrhoea*; all suspected cases must be confirmed by culture.

To detect *T. vaginalis* if a bacterial culture is not possible, a fresh dark field microscopic examination should be performed. In this case, the secretion should be collected with a swab and placed in a tube containing 1 ml of saline solution kept at 37°C. The fresh slide must be examined immediately to avoid the disappearance of Trichomonas motility that, together with the morphology, is one of its specific features. Culture is a more sensitive method of detection than the microscopic analysis of trophozoites.

The microbiological culture examination of the urethral swabs aims to detect aerobic bacteria, fungi, *N. gonorrhoeae*, *G. vaginalis* and urogenital mycoplasmas (Table 3).

To detect *U. urealyticum* and *M. hominis*, the buffer must be dissolved in a transport/culture medium. Three drops of this medium should be seeded on solid A7 medium (Vázquez *et al.*, 1995), which will be incubated at 37°C for 48–72 h in anaerobic or microaerophilic conditions. To set up a possible antibiogram, the test tubes containing the remaining medium can be refrigerated for a few days or frozen at −80°C.

Bacterial culture is the ‘gold standard’ to detect *T. vaginalis* both for sensitivity and specificity. The exudate, collected with a sterile swab, should be sown immediately after collection in 5 ml CPLM Trichomonas broth (Patel *et al.*, 2000) preheated to 37°C containing streptomycin (1000 mg ml⁻¹), penicillin (1000 U ml⁻¹), chloramphenicol (50 g ml⁻¹) and sterile horse serum (50 ml 1⁻¹). Incubation is performed at 37°C for 5 days with fresh microscopic observations performed daily, beginning on the second day of incubation.

Anaerobic micro-organisms should not be routinely sought out, but other micro-organisms observed during Gram staining in the setting of an increase in polymorphonuclear cells and associated symptoms (*Haemophilus* species, *Streptococcus* species, etc.) should be investigated.

A further culture method involves the use of a gallery with domes that contain substrates for micro-organism identification as well as lyophilized antibiotics. These domes are rehydrated with the transport/culture medium which has been inoculated with the sample that has been tested. The domes are then covered with paraffin oil to ensure anaerobic conditions and incubated at 37°C for 24 h. Cultures in liquid medium provide better results than those sown directly on solid medium (due to the dilution of potential inhibitors and the breaking of immune complexes, etc.). PCR can also be used to specifically highlight genital mycoplasmas that are difficult to cultivate, such as *Mycoplasma genitalium* and *Mycoplasma fermentans*.

**Identification test and sub-analysis of specific pathogens.** *N. gonorrhoeae* grows on Thayer–Martin agar medium after 24–48 h incubation. It forms small, translucent, grey–white, cytochrome oxidase-positive colonies. Gram staining shows characteristic Gram-negative diplococci. These features allow presumptive identification of *N. gonorrhoeae*, but tests are still necessary to confirm the identification. Along with an antibiogram, it is essential to perform the nitrocefin test to detect β-lactamase production.

*G. vaginalis* grows on media containing human blood and it forms small, catalase-negative colonies surrounded by a β-haemolysis halo. The identification is based on morphological/ tinctorial features (Gram-variable cocci). A sufficient confirmatory test is the hippurate hydrolysis test which is carried out as follows: (a) add 0.4 ml sterile distilled water to a test tube and then add a disk of blotting paper soaked in sodium hippurate; (b) add a large loopful of the bacterial culture under examination and stir to form a homogeneous suspension; (c) incubate the test tube for 2 h in a thermostatically controlled water bath set at 37°C; (d) followinc incubation, add 0.2 ml ninhydrin (in a 3.5% solution of acetone/butanol) and shake gently; and (e) incubate the test tube for 15 min and read the colorimetric reaction. The appearance of a blue–purple colour indicates that hippuricase production has occurred and that the test is positive.

Fungi, specifically *Candida albicans* must be sought. An extremely simple method for recognizing this fungus is the germ-tube test or filamentation test, although it has been demonstrated that 5% of *C. albicans* subtypes may produce a negative result. The test is carried out as follows: (a) dissolve five or six colonies grown on Sabouraud agar in a tube containing 1 ml serum and (b) incubate the tube for 4 h at 37°C and proceed to microscopic examination. The presence of germ-tubes (small filaments that radiate from the blastospore surface) allows the identification of the *C. albicans* species (Lipperheide et al., 1993). Chromogenic media can be used to identify other species; these media allow quick distinction between the most common species based on the colour of the colonies or by tests based on sugar assimilation and resistance to actidione. Currently,

**Table 3. Microbiological cultural examination of urethral swabs**

Incubation temperature was 37°C for all cultures.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Culture medium</th>
<th>Incubation conditions</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic bacteria</td>
<td>Blood agar</td>
<td>Aerobiosis</td>
<td>24 h</td>
</tr>
<tr>
<td>Fungi</td>
<td>Sabouraud agar</td>
<td>Aerobiosis</td>
<td>48 h</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Thayer–Martin agar</td>
<td>5% CO₂</td>
<td>48 h</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>Gardnerella agar</td>
<td>5% CO₂</td>
<td>48 h</td>
</tr>
</tbody>
</table>
miniaturized systems composed of microgalleries that contain the dried substrates for assay are commercially available. Antifungal susceptibility testing should be performed only in the presence of recurrent infections that are particularly difficult to eradicate.

Urogenital mycoplasma identification is based on colony morphology and metabolic properties (the ability to hydrolyse urea or arginine and the ability to ferment glucose). The size and appearance of the colonies on solid A7 medium are extremely variable; in fact, there may be colonies on the same plate that have variable diameters ranging from 10 to 500 μm (they must be observed under a microscope) or with uniform or irregular appearances. The colonies are found on the cell edges and in the periphery of the inoculum drop. Colonies of M. hominis may appear in two different forms. In the first, a central part that is optically denser and is the true germinal centre of the colony appears more or less grainy and is surrounded by the second type, which is a peripheral translucent ring that gives the colony the appearance of a fried egg. The second type forms later and it can be very thin if the number of colonies is particularly high. U. urealyticum forms smaller colonies than M. hominis and this area is visible only as the central darker part, or as the ‘egg yolk’. Solid A7 medium contains urea and manganese sulfate, and due to ammonia liberation by urea hydrolysis, manganese oxide is formed which gives a dark brown–black coloration to the colonies and makes them easily identifiable, as they have the appearance of a sea hedgehog.

The tunnel culture system is used for mycoplasmas for counting, identification and determination of antibiotic resistance. The system is based on the ability of U. urealyticum and M. hominis to metabolize urea and arginine, respectively. Their growth in liquid medium is indicated by a colour change of phenol red from yellow–orange to pink–fuchsia, which reflects the alkalization of the culture broth. Samples that contain a very high number of bacteria can cause a colour change in all gallery wells. In this case, the sample should be diluted. The bacterial counts [unified code count (UCC) ml⁻¹] are based on an enzyme kinetics principle for which the rate of urea or arginine hydrolysis by U. urealyticum and M. hominis, respectively, is proportional to the quantity of micro-organisms in the sample. Among antibiotics included in the system, three (lincomycin, trimethoprim/sulfamethoxazole and erythromycin) are identifiers, while the other three (dosycycline, ofloxacin and roxithromycin) may indicate the emergence of resistance. The emergence of strains resistant to macrolides and cyclins, especially in cases of non-eradication, makes the antibiogram indicative (>100 000 UFC). This approach can only be realized with liquid media in type antibiotic galleries or in microplates. A reaction involving enzyme inhibition is used; when the germ is sensitive to the antibiotic in the pit, its metabolism is inhibited and the medium, which contains phenol red as an indicator, does not change colour. If the germ is resistant, it grows and the medium turns red.

C. trachomatis is mainly detected using cell culture, which is the reference technique. Other common techniques are direct immunofluorescence, enzyme immunoassay and hybridization by probes and genomic amplification methods [PCR, ligase chain reaction (LCR), strand displacement amplification (SDA) and transcription-mediated amplification (TMA)].

Immunofluorescence uses monoclonal antibodies directed to the main outer-membrane proteins and against the lipopolysaccharide of the outer membrane of C. trachomatis. This method highlights the extracellular elementary bodies (300 nm, perfectly rounded with bright apple-green fluorescence). The sample is smeared on a glass slide for fluorescence, and after fixation with acetone, it is placed in contact with the monoclonal antibody. After incubation at room temperature for 30 min, the sample is observed under a fluorescence microscope. The advantage of this technique is that it allows a rapid diagnosis, even though it requires specialized personnel who are able to discriminate possible artefacts of the preparation, and it is the only technique that allows the assessment of the suitability of the preparation. Indeed, in the absence of epithelial cells, a negative result for the specimen should not be considered. False positives can be reduced by using two monoclonal antibodies. This technique’s sensitivity is high enough when you consider the presence of two elementary bodies as a positive/negative cut-off.

An advantage of the immunoassays is the ability to provide a diagnosis in a few hours. Additionally, they have a sensitivity comparable to cell cultures but do not have the risks associated with contamination. These methods have shown sufficient sensitivity and good specificity for cell cultures but do not have the risks associated with contamination. These methods have shown sufficient sensitivity and good specificity for urethral swabs, though their use for urine is limited by poor sensitivity, especially among asymptomatic subjects in whom there are often a low number of micro-organisms.

Hybridization is a molecular biological technique that allows the identification of a micro-organism using specific nucleic acid probes. This method is highly sensitive (85–100 %) and specific (98–100 %) and also allows for the identification of a small number of copies of bacterial DNA. Compared to this method, cell culture has a sensitivity of 70–85 % and the enzymic methods have sensitivities of 60–80 %. The advantages of hybridization are their high sensitivity, specificity and the ability to perform the test on a urine sample, which is a non-invasive method compared with a urethral swab. The disadvantages are related to its inability to differentiate live micro-organisms from dead ones and its relative complexity. The limitations of these methods related to their high cost, relative technological complexity and the possibility of false positives were resolved by standardization and the introduction of a low-cost test that allowed reduced manual dexterity. The main methods used are described below (Jensen et al., 2003; Murray et al., 2003; Scottish Intercollegiate Guideline Network, 2009).

PCR is a molecular biological technique that allows multiplication (amplification) of nucleic acid fragments with known initial and terminal nucleotide sequences. PCR amplification produces a considerable amount of genetic material in vitro in a short amount of time. This method is based on the ability of denatured single-chain nucleic acids to specifically pair with complementary sequences of synthetic DNA (primers) that trigger and direct the synthesis of a complementary chain by means of DNA polymerase. The number of DNA target molecules doubles for each PCR cycle and they can then be detected with conventional methods. For LCR, the target is located within the plasmid DNA of C. trachomatis. This is a short sequence that is highly conserved in all serotypes. The LCR can be used for the diagnosis of C. trachomatis infection in urethral swabs or urine samples, making non-invasive screening possible in selected populations. Sampling is performed with the kit provided by the manufacturing company. Before analysis, the samples can be stored for 4 days at room temperature or at −20 °C for 60 days.

SDA allows the performance of DNA fragment amplification cycles in isothermal conditions (52 °C), as well as the ability to detect successful amplification in real-time. In this method, DNA polymerase can simultaneously synthesize new DNA by copying the mould and can move the complementary DNA strand synthesized in the previous cycle.

TMA (Gen-Probe) uses rRNA as a target, the hybridization protection assay for detection and TMA for amplification. Using the rRNA target, the test is very sensitive because the rRNA is present in thousands of copies in bacterial cells. The sensitivity is further increased due to the hybridization protection assay method, which exploits the high sensitivity of chemiluminescence. The TMA amplification is isothermal, achievable in any incubator, sufficiently sensitive to detect a single copy of a DNA or RNA target in a clinical sample and has very
fast kinetics. The main advantages of this method are greater sensitivity using the abundant rRNA as a target, exponential amplification at a single temperature and the performance of all test procedures in one test tube without washing processes. This method further minimizes the risk of contamination and false positives.

An antibiogram should be produced by testing any Gram-positive or Gram-negative aerobic organism present in pure culture for chemosensitivity to antibiotics.

**Meares–Stamey test**

The Meares–Stamey test has been developed to diagnose chronic bacterial prostatitis. It requires three urine samples to be collected and examined separately. The last sample is taken after prostate massage. The test is able to discriminate between chronic bacterial prostatitis and complicated lower UTI by identifying the source (urethra, bladder, prostate) through a sequential and fractionated collection of urine and secretion obtained after prostatic massage.

**Indications.** The Meares–Stamey test is indicated when one or more of the following conditions occur:

- the presence of one or more of the following recurring symptoms (associated with prostatitis): onset in the post-ejaculatory phase of prostate-perineal, scrotal-testicular, penile, vesical or suprapubic tenesmus (Zermann et al., 1999); prostatorrhoea, often associated with voiding disorders and occasional haematospermia;
- sperm culture with repeated negative results;
- persistent leukocytospermia, even after antibiotic therapy;
- ultrasonographic findings of prostate-vesiculitis (prostatic lobe asymmetry, multiple hypoechoic areas (acinar stagnation)); vesicular anteroposterior diameter (>14 mm) with polycyclic areas resistant to first therapeutic measures.

**Contraindications.** The Meares–Stamey test should not be performed in cases of acute prostatitis (acute infection, abscess-like), which are almost always due to a bacterial infection, and the pathogens involved are typical of the micro-organisms of the urinary tract.

**Sampling procedure.** The patient should have a full bladder, must not have ejaculated for 2 days and must not have taken antibiotics in the previous month. The patient should collect the first 10 ml of urine (VB1) and then another 10 ml of urine (VB2). At this point, a prolonged prostate massage is performed at both gland lobes and the prostatic secretion is collected by trying to facilitate the escape from the urethral meatus by pressing the penis from the base to the glans (EPS). The patient should then collect another 10 ml of urine (VB3) and thereafter, he can empty his bladder completely.

**Microscopic examination.** A drop of EPS is subjected to Giemsa staining. The presence of more than ten leukocytes per microscopic field (× 400 magnification) and/or the presence of leucocyte aggregates is indicative of prostatitis. A bacterial count in EPS and VB3 at least ten times higher than in VB1 and VB2 suggests that the germs are of prostatic origin. A higher load in VB2 than in VB3 suggests that bacteriuria should be treated with drugs such as nitrofurantoin that do not penetrate to the prostate. The test should then be repeated.

**Bacterial culture.** Samples should be sown in the shortest possible time on selective and non-selective media after 1:100 dilution in sterile saline solution and inoculation of 100 μl by spoutation. To detect fungi, aerobic organisms, G. vaginalis and urogenital mycoplasmas, the procedures for seminal culture should be performed.

**Identification, antibiogram and C. trachomatis research.** The procedures for seminal culture should be performed.

**Swab from balanopreputial sulcus**

**Indications.** This investigation is useful for integrating the urethral swab, but has limited indications in patients with a history/clinical presentation of the following: phimosis, hypospadias, diabetes mellitus, recurring balanoposthitis and persistent UTI. Patients with balanitis exhibit glans irritation and inflammation, which is often extended to the foreskin (balano-). Due to the high frequency of occasional balanitis, irritation due to the simple mechanical effects of maceration and poor sanitation in the balanopreputial region should be excluded first. After exclusion of these forms of balanitis, other cases worthy of further investigation are:

- (a) cases in the purulent stage, where the balanitis may be secondary to urethral infection that may go unnoticed (these cases of balanitis are often located at the meatus). It is useful to take into consideration the following micro-organisms: N. gonorrhoeae, mycoplasmas, T. vaginalis, G. vaginalis, C. albicans, group A and B Streptococcus and S. aureus (Horner & Taylor-Robinson, 2011);
- (b) balanitis associated with skin (condylomata acuminata or plana) or erythematous-vesicular lesions. In these cases, the organisms responsible for balanitis that should be investigated are Herpes simplex virus (HSV-1, HSV-2) and HPV.

**Sampling procedure.** Sampling should be performed with nylon plugs from the balanopreputial sulcus. Many swabs should be used as there are different types of tests to be performed. The patient should not use antibiotics or antifungal topical or systemic therapy for 3–4 days prior to the test. Samples should be stored and transported as described for the urethral swab.

**Bacterial culture.** For the cultivation of fungi, aerobic organisms, N. gonorrhoeae and G. vaginalis, the material is seeded and incubated. The culture media used for U. urealyticum and M. hominis are the same as those described for the urethral swabs.

**Characterization of patients with HPV infection**

The clinical management of HPV infections and the prevention of HPV-related diseases have undergone substantial changes in recent years because of the following three factors.

1. Molecular biology has developed very sensitive and specific methods to detect viral DNA. This allows the diagnosis of HPV infection even before obvious clinical manifestations develop. HPV-related diseases in men have been given little attention. Indeed, the most frequent clinical manifestations (warts) are considered an expression of low clinical relevance compared to HPV-related lesions of the cervix in women.

2. The availability of HPV vaccines able to prevent HPV-related clinical manifestations is of great relevance. Vaccination is done to prevent cervical cancer in almost all countries including Italy where the National Health System provides this opportunity to young women.

3. The HPV-related symptoms in men comprise mucosal and/or skin lesions detected on clinical
examination. Foreskin, penis, perianal region, oral cavity, oropharynx and larynx lesions may be asymptomatic or rarely painful (penis, prepuce, perianal region); they may also be responsible for local pain (oral cavity, oropharynx) or pain and functional impotence (larynx). The most common symptomatic manifestations are the genital (anogenital) and oral warts, laryngeal papillomas and infertility.

It is widely recognized that the following categories of men should undergo HPV testing: (a) those with HPV-related symptoms; (b) partners of women who are HPV-positive or with HPV-related diseases; and (c) subjects with numerous partners.

Other possible indications are: (a) other men at risk such as those who are HIV-positive or who have HIV-induced immunosuppression or who are homosexual; (b) idiopathic asthenozoospermia; (c) before sperm cryopreservation; (d) before assisted reproductive technology; and (e) in couples with recurrent miscarriage.

The fundamental clinical approach to ascertain the presence of warts is penoscopy which may be implemented by the application of acetic acid. In this case, penoscopy not only allows a more careful characterization of exophytic warts, but also identifies less evident lesions which can completely escape the clinical examination. However, the white colour observed after the addition of acetic acid is not specific for HPV lesions. Several factors such as traumatic irritation, candidiasis or other micro-organism-related inflammatory conditions (Chlamydia, Mycoplasma, etc.) may cause it.

The molecular diagnosis is based on DNA amplification. Hybrid capture 2 (HC2, Digene), widely used to diagnose HPV infection in women, has been validated in samples of cervical cells but not in anogenital samples taken from different sites in men. The samples of exfoliated cells can be collected with Dacron swabs or buds, moistened with saline or with a cytobrush or other similar devices. Several tests can be used for the detection of HPV in the male. These tests are based on the extraction of nucleic acids from the cytological sample, on the amplification of a sequence of HPV by PCR with degenerate primers or consensus and on the detection of the amplification by hybridization with probes, or with reverse hybridization on nitrocellulose strips or strips of nylon or microchip, or by gene sequencing. The latter has, however, low sensitivity in the case of multiple infections (Pakendorf et al., 1998; Foresta et al., 2011; Garolla et al., 2011).

Conclusions and recommendations for future research

There are several important clinical variables that may affect the biological consequences of the male genital tract inflammation on sperm parameters. These include the type of initiating factor, the duration of inflammatory mediator activity and the initial condition of the antioxidant system in the semen. Moreover, there are two other issues that are frequently underestimated or not commonly evaluated in clinical practice, which are the anatomical extension of the inflammatory process and lymphocyte subpopulations in the semen.

With respect to the anatomical extension, we have shown that among men with MAGI, those with prostatitis show better sperm parameters than those with prostate-vesiculitis and prostate-vesiculo-epididymitis. The latter have the poorest sperm quality. Ultrasound examination represents an excellent diagnostic tool to evaluate the site of inflammation with objective criteria to support the diagnosis (La Vignera et al., 2012b). The low reproducibility of ultrasound abnormalities may be overcome by proper training of the operator and with close integration between ultrasonic signs and laboratory markers. This represents an interesting area of future research.

Another overlooked aspect of semen inflammation concerns leukocytes. Currently, the WHO recommends that polymorphonuclear leukocytes in the semen should be evaluated as a first level diagnostic evaluation using assays that recognize the peroxidase enzyme. However, chronic inflammation can be associated with an apparent reduction in leukocytes but an increase in the concentration of lymphocytes detectable in the semen through unconventional methods (e.g. flow cytometry) using monoclonal antibodies specific for lymphocyte subpopulations (CD45, CD4, CD8, etc.). We believe that this parameter is particularly important since it may cause an incorrect evaluation of the disappearance of inflammation and an inadequate treatment length.

Finally, we consider the problem of the individual susceptibility to chronic inflammation. Future research into this issue should focus on antimicrobial resistance to chemotherapy, the role of the Toll-like receptor and structural changes in the prostate associated with recurrent prostatitis.

With respect to antibiotic resistance, the poor antibiotic penetration rate within the prostate, the resistance to drugs by uropathogens, the adverse events associated with antibiotic treatment, the persistence of prostatic calculi and biofilm formation in the prostate gland are all factors that contribute towards decreasing the eradication rate of chronic bacterial prostatitis (Letkiewicz et al., 2010). The selection of the optimal antimicrobial agent must take into account patient-specific factors, such as the characteristics of the infection (severity), the local resistance pattern, pharmacokinetic and pharmacodynamic principles and cost. Fluoroquinolones are the first choice of treatment for chronic bacterial prostatitis because of their favourable pharmacokinetic properties at the site of infection (Wagenlehner & Naber, 2006).

Toll-like receptor 4 (TLR4) is considered to be a major sensor of danger signals and a key trigger of the innate immune response. TLRs have also been implicated in the
development of different inflammatory diseases in organs in which epithelial-stromal interactions are critical for homeostasis. A recent study suggested that the prostate is able to recognize pathogens and to initiate immune responses. In addition, TLR4 appears to be implicated in the vital stromal-epithelial interactions that maintain prostate homeostasis during prostatitis, as well as those following androgen deprivation (Quintar et al., 2006). Interactions between the immune system and the reproductive system have important consequences for fertility and reproductive health in general. There is increasing evidence that many of the interactions between the immune and reproductive systems involve TLRs. While there is no doubt that TLRs are important in providing protection against infection in the reproductive tract, there is also increasing evidence for the involvement of TLRs in the basic pathology and physiology of reproduction (Girling & Hedger, 2007).

Finally, we evaluated the morphological abnormalities in the prostate and seminal vesicles of patients with MAGI who failed to respond to antibiotic treatment. The results of this study showed that the major ultrasound characteristics associated with the failure to respond to antibiotics are bilaterally extended prostate-vesiculitis and prostatic-vesiculitis with unilateral or bilateral subobstruction of the ejaculatory ducts (La Vignera et al., 2008). In a more recent study we showed that patients with microbiological persistence or persistence plus superinfection showed a higher prevalence of complicated forms of MAGI (prostate-vesiculitis and prostate-vesiculo-epididymitis) than did patients with microbiological eradication or eradication with superinfection (La Vignera et al., 2012c). Fig. 1 shows a proposed microbiological algorithm to be used in patients with semen inflammation.

**Fig. 1. Original algorithm for the evaluation of patients with inflammation of the semen.**

![diagram](image-url)

### References


