

REVIEW ARTICLE

Male accessory gland infection and sperm parameters (review)

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Summary

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Male accessory gland infection (MAGI) has been identified among those diagnostic categories which have a negative impact on the reproductive function and fertility in males (Rowe *et al.*, World Health Organization Manual for the Standardised Investigation and Diagnosis of the Infertile Couple, Cambridge University Press, Cambridge, 1993). MAGI is a hypernym which groups the following different clinical categories: prostatitis, prostate-vesiculitis and prostate-vesiculo-epididymitis. Some of the characteristics they share are: common diseases, mainly have a chronic course, rarely cause obstruction of the seminal pathways, can have an unpredictable intracanicular spread to one or more sexual accessory glands of the reproductive tract, as well as to one or both sides. In this review, we show that all components involving the inflammatory response (from the agents which first trigger it to each component of the inflammatory response dynamic) can deteriorate conventional and/or non-conventional sperm parameters arising from one or more of the following mechanisms: altered secretory function of the epididymis, seminal vesicles, and prostate which reduce the antioxidant properties or scavenging role of the seminal plasma; deterioration of spermatogenesis; and (unilateral or bilateral) organic or functional sub-obstruction of the seminal tract.

Male accessory gland infection and sperm output

Male accessory gland infection (MAGI) has been identified among those diagnostic categories which have a negative impact on the reproductive function and fertility in males. According to the Rowe *et al.*, (1993), MAGI is diagnosed when abnormal sperm parameters are found associated with at least one factor A plus one factor B, one factor A plus one factor C, one factor B plus one factor C or two factors C (Table 1).

Male accessory gland infection involves different clinical categories such as prostatitis, prostate-vesiculitis and prostate-vesiculo-epididymitis (PVE) which share the following characteristics: they are frequent diseases, mostly with a chronic course, rarely cause obstruction of the seminal pathways, can have an unpredictable intracanicular spread to one or more sexual accessory glands of the reproductive tract and can effect either one side or both

(Vicari *et al.*, 2006b). With the aid of scrotal and trans-rectal prostate-vesicular ultrasound scans, MAGI may be classified in: (i) uncomplicated form: including prostatitis itself, and (ii) complicated forms, which encounter the inflammatory involvement of both prostate and seminal vesicles (prostate-vesiculitis) or the involvement of all three glands (PVE).

A debate has been going on to establish whether MAGI can alter sperm parameters. A voluminous collection of literature suggests that MAGI may negatively interfere with sperm quality in many ways. The inflammatory response leads to the negative impact on sperm function as many inflammatory mediators released in higher amounts during MAGI have a detrimental effect of germ cells (Agarwal *et al.*, 2003; Sanocka *et al.*, 2003). These include reactive oxygen species (ROS) and cytokines (Ochsendorf, 1999; Vicari, 2000; Vicari & Calogero, 2001; Vicari *et al.*, 2002; Weidner *et al.*, 2002; Diemer *et al.*,

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

Factor	Description
A	<i>History:</i> positive for urinary infection, epididymitis and/or sexually transmitted disease <i>Physical signs:</i> thickened or tender epididymis, tender vas deferens and/or abnormal digital rectal examination
B	<i>Prostatic fluid:</i> abnormal prostate fluid expression and/or abnormal urine after prostatic massage
C	<i>Ejaculate signs:</i> leucocyte >1 mil/mL, culture with significant growth of pathogenic bacteria, abnormal appearance, increased viscosity, increased pH and/or abnormal biochemistry of the seminal plasma

2003a) which may persist even after successful treatment with antimicrobials. As a matter of fact, the antioxidant capacity of seminal plasma is progressively exhausted and cannot be restored as male accessory glands are often dysfunctional. As a matter of fact, ultrasound abnormalities have been found in the accessory glands (prostate, seminal vesicles and/or epididymis) of infertile patients with MAGI and elevated bacteriospermia ($\geq 10^5$ CFU/mL) or with *Chlamydia (C.) trachomatis* or *Ureaplasma (U.) urealiticum* infection (Vicari, 1999; Vicari *et al.*, 2006b). These patients also have an increased inflammatory response and an impaired semen quality which is directly related to the extension of MAGI (Vicari, 1999; Bayasgala *et al.*, 2004). In recent times, we also reported that patients with bilateral PVE have poorer sperm parameters compared with patients with an unilateral involvement (Vicari *et al.*, 2006b).

Several patho-physiological mechanisms may impair sperm function during MAGI, including reduction of the accessory gland function, obstruction of sperm transport and dysregulation of spermatogenesis (Purvis & Christiansen, 1993; Comphaire *et al.*, 1999). Innate host defence mechanisms which are called into play to overcome the infection comprise neutrophil infiltration and secretion of their products, such as ROS and cytokines, and at a later chronic inflammation stage, the kinetics of other cellular mediators, such as the epididymal macrophages, testicular dendritic cells (DCs) and post-infectious/inflammatory secretory abnormalities. All these factors play a significant role because they are critical for the defence against potentially harmful microorganisms, but their excessive and inappropriate activation significantly contributes to tissue damage and worsening of the disease (Meinhardt & Hedger, 2011).

In this article, we are going to review the effects of: (i) microorganisms, (ii) viruses, (iii) ROS overproduction, (iv) the main pro-inflammatory cytokines, (v) epididymal macrophages and dendritic cells, and (vi) the post-infective secretory alterations.

Effects of microorganisms

Several kinds of microorganisms can be found in the male urogenital tract which are associated with sperm parameter abnormalities, especially motility and mitochondrial sperm function and/or chromatin/DNA integrity.

The major difficulty in interpreting microbiological findings is the presence of contaminating microbes or of inhibitory substances known to be present in the prostatic secretions, as well as previous courses of antibiotics. Thus, the pathogenetic involvement of a given microorganism should be confirmed through quantitative bacteriological cultures in the semen (growth of $>10^3$ pathogenic bacteria or $>10^4$ non-pathogenic bacteria in seminal plasma diluted 1 : 2 with saline solution) (Comhaire *et al.*, 1980) or four (Meares & Stamey, 1968) and/or two (Nickel, 1997) glass tests.

Escherichia coli

Many studies have analysed the effects of *E. coli* on sperm function mainly using an in vitro approach. Uropathogenic *E. coli* serotype 06 inhibited significantly the progressive motility of normal spermatozoa separated by swim-up; an effect abolished by the addition of chloramphenicol in the incubation medium. On the other hand, no effect on sperm motility was observed after incubation with *E. coli* culture filtrates. Electron microscopy analysis revealed multiple adhesions of *E. coli* to spermatozoa (Diemer *et al.*, 1996). An inhibitory effect of *E. coli*, but not of the enterococcus, on sperm motility was subsequently confirmed by the same group of researchers (Huwe *et al.*, 1998). The co-incubation of normal spermatozoa with *E. coli* and polymorphonuclear (PMN) has been reported to reduce sperm motility parameters evaluated by computer-assisted sperm analysis (CASA) more profoundly than when spermatozoa were incubated with PMN alone, suggesting that *E. coli* is the primary agents that interfere with sperm motility (Diemer *et al.*, 2003b).

Normal spermatozoa incubated with *E. coli* resulted in an higher percentage of spermatozoa with phosphatidylserine (PS) externalization (a marker of early apoptosis) and with apoptosis/necrosis (annexin V-FITC-positive/propidium iodide-positive), whereas the incubation with phorbol-12-myristate-13-acetate activated PMN only showed a small increase in apoptosis/necrosis (Villegas *et al.*, 2005). These results suggest that *E. coli* is directly capable of altering ejaculated sperm function without involving any of the molecular mechanisms which alter their motility, vitality and DNA integrity. Therefore, incubation with *E. coli* lowered the percentage of spermatozoa having an elevated mitochondrial membrane potential

(MMP); this finding was associated with decreased sperm motility and viability. ROS production and PS externalization did not change significantly. Interestingly, a similar effect was observed incubating spermatozoa with the supernatant from *E. coli* culture, suggesting the soluble factors damage sperm function (Schulz *et al.*, 2009).

In recent times, in the attempt to understand the mechanism by which *E. coli* inhibits sperm motility, Prabha *et al.* isolated and purified the factor responsible for such an effect and they named it sperm immobilization factor (SIF). SIF is a 56 kDa molecule which causes instant immobilization without agglutination of human spermatozoa at a concentration of about 1 mg/mL and death at a concentration of about 2 mg/mL. Spermatozoa incubated with SIF revealed multiple and profound alterations involving all superficial structures of spermatozoa as observed during electron microscopy (Prabha *et al.*, 2010).

Neisseria gonorrhoeae

Few studies have explored the effects of *Neisseria (N.) gonorrhoeae* on sperm parameters. Liu *et al.* reported that this microorganism did not significantly modify the motility parameters of normal spermatozoa evaluated by CASA. On the other hand, using the same experimental model, *Staphylococcus aureus* significantly decreased sperm motility and viability (Liu *et al.*, 2002). Interestingly, *N. gonorrhoeae* up-regulates several anti-apoptotic mechanisms on the urethral epithelium and protects these host cells from in vitro staurosporine exposure-induced death. These *N. gonorrhoeae*-induced anti-apoptotic effects may represent a mechanism put into action by this microorganism to survive and proliferate in the host epithelium (Binnicker *et al.*, 2003). It is not known whether a similar mechanism is also exerted on germ cells.

Chlamydia trachomatis

Chlamydia trachomatis infection may cause sperm apoptosis because the number of spermatozoa with fragmented DNA has been reported to be higher in patients with chlamydial infection compared with control patients (Wan *et al.*, 2003). Asymptomatic men with chlamydial infection have a significantly higher number of leucocytes and a higher ejaculate volume than those whose ejaculate did not show any chlamydial infection evaluated using PCR analysis. No significant differences were observed for the rest of the parameters (Hosseinzadeh *et al.*, 2004). By contrast, sperm concentration, motility and morphology were significantly worse in men with both chlamydial and mycoplasma infection, whereas sperm viability was not significantly affected. Interestingly, these patients had also

an increased percentage of spermatozoa with DNA fragmentation which decreased after antibiotic administration (Gallegos *et al.*, 2008).

Ultrastructural examination suggested that the presence of abnormal spermatozoa during chlamydial infection may relate to the microorganism per se or to the host immune/inflammatory response. In addition, bacteria were detected in the seminal leucocytes suggesting that this intracellular persistence may be responsible for the establishment of a latent or chronic infection (Gallegos-Avila *et al.*, 2009). Chlamydial infection has recently been found associated with a significantly higher pH and seminal leucocyte number as well as a significantly lower percentage progressive motile spermatozoa in infertile patients compared with fertile men with chlamydial infection. This was associated with higher seminal plasma IL-6 and IL-8 concentrations (Kokab *et al.*, 2010).

Mechanism(s) through which *C. trachomatis* alters sperm function has/have been deeply explored. An in vitro model showed that *C. trachomatis* serovar E elementary bodies (EB) incubated with spermatozoa of normal men decreased significantly sperm motility and viability, whereas serovar LGV EB decreased only sperm viability. The co-incubation with dead EB did not have any effect on these parameters, suggesting that it requires an alive microorganism (Hosseinzadeh *et al.*, 2001). Subsequently, it was shown that the lipopolysaccharide (LPS) extracted from *C. trachomatis* EB had the same effects of alive EBs (Hosseinzadeh *et al.*, 2003). In addition, LPS was shown to cause apoptosis when incubated in vitro with normal spermatozoa, an effect mediated by caspase 3 (Eley *et al.*, 2005). Hakimi *et al.* showed that the lipid A and the 3-deoxy-D-manno-octulosonic acid, toxic components of the *C. trachomatis* LPS, have spermicidal effects similar to LPS and that both molecules cause sperm apoptosis with a mechanism caspase-mediated (Hakimi *et al.*, 2006). We have investigated the effects of *C. trachomatis* on sperm apoptosis by incubating spermatozoa from normozoospermic healthy men with increasing concentrations of *C. trachomatis* serovar E EBs. After 6 h of incubation, *C. trachomatis* did not have any effect on the percentage of spermatozoa with PS externalization, whereas the number of spermatozoa with this abnormality increased significantly after 24 h of incubation. Sperm DNA fragmentation increased significantly after both 6 and 24 h of incubation (Satta *et al.*, 2006).

Ureaplasma urealyticum

Ureaplasma urealyticum is the most common microorganism found in infertile men with a prevalence of 10–40% (for review see Dieterle, 2008). The presence of *U. urealyticum* in the human male genital tract has

been found associated with a significantly reduced sperm concentration, whereas no effect has been reported on semen volume and sperm motility, viability or morphology (Upadhyaya et al., 1984). A stronger effect on sperm parameters was later reported in infertile men with genital tract infection, including decreased semen volume, sperm concentration, motility, morphology and viability. However, this study does not allow to identify specifically the effects of *U. urealyticum* on sperm parameters because these end-points have been reported regardless of the aetiology of the infection (Sanocka-Maciejewska et al., 2005). In patients with isolated *U. urealyticum* infection, Wang et al. found an altered semen viscosity, pH value, and sperm concentration, whereas all the other parameters were not affected significantly (Wang et al., 2006). Altogether these findings suggest that *U. urealyticum* reduces sperm concentration, but does not have a relevant effect on the other conventional sperm parameters. However, Zheng et al. reported that infertile Chinese men with a decreased sperm concentration showed significantly lower sperm motility and viability compared with patients without *U. urealyticum* infection. These effects on motility were associated with lower seminal plasma α -glucosidase levels, whereas seminal plasma acid phosphatase and fructose were unaltered, suggesting a possible epididymal site of action (Zheng et al., 2008). A reduced sperm reaction to acrosome induction has also been reported in vivo in men with *U. urealyticum* infection returned to normal after antibiotic treatment in most patients. This effect seems to be *U. urealyticum* specific, as *Mycoplasma (M.) hominis* has no in vivo effects (Köhn et al., 1998).

Sperm motility and the percentage of normally shaped spermatozoa, hyperactivation and calcium ionophore-induced acrosome reaction decreased significantly after an overnight incubation with *U. urealyticum* (Rose & Scott, 1994). *U. urealyticum* seems to have a dual effect on sperm motility, increasing it after a short-term incubation (45 min) and decreasing sperm motility after 4 h. This apparent discrepancy may relate to the glycolysis stimulation initially induced by *U. urealyticum*, later followed by the exhaustion of mitochondrion-produced energy consumed by the germ (Núñez-Calonge et al., 1998).

Ureaplasma urealyticum serotype 4 was the most effective in reducing the Hamster's oocyte sperm penetration rate compared with other mycoplasmas. As the number of spermatozoa adsorbed to Hamster's oocytes was not influenced by *Mycoplasma* preincubation. This suggests that the inhibition of penetration is not attributed to a masking of sperm membrane sites (Busolo & Zanchetta, 1984; Soffer et al., 1990).

Interestingly, the infection with *U. urealyticum* has also been reported able to alter the concentration of microelements in the seminal fluid of infertile patients. In fact,

patients with *U. urealyticum* had an increased ratios Cu/Zn and Cd/Zn and of the concentrations of As and Mg in the seminal fluid (Wang et al., 2005). These abnormalities may contribute to the reduction of the sperm quality found by some authors.

It is noteworthy to recall that *Mycoplasma* infection may alter glycolipid metabolism in the early primary spermatocytes. Particularly, these microorganisms may desulphate sulfo-galactosyl-glycerolipid (SGG), an important molecule for the sperm-egg binding. Therefore, this mechanism may contribute to the negative impact of *U. urealyticum* infection on human fertility (Ma & Xu, 2004). Furthermore, the presence of *U. urealyticum* may affect negatively the implantation of the embryo (Dieterle, 2008).

To obtain further data concerning the effects of *U. urealyticum* on sperm function, non-conventional sperm parameters have also been studied. Shang et al. found that patients with *U. urealyticum* infection have an increased number of spermatozoa with fragmented DNA, evaluated by TUNEL assay, compared with control patients (Shang et al., 1999). This has been confirmed by a later study which also reported an increased percentage of spermatozoa with less stable chromatin. After treatment with doxycycline, a significant improvement of both parameters was observed. Accordingly, spermatozoa incubated with *U. urealyticum* showed a significant dose- and time-dependent chromatin decondensation and DNA damage. The percentage of human spermatozoa with denatured DNA increased by almost 50% after 30 min of incubation with the serotypes 3 and 8, at a concentration of 100 ureaplasmas/spermatozoon compared with uninfected control spermatozoa (Reichart et al., 2000). A study on experimentally infected with *U. urealyticum* male rats (serotype 8) showed an increased number of TUNEL-positive cells and areas in the testis and a Fas-FasL overexpression in germinal and Sertoli cells. These findings show that *U. urealyticum* increases germ cell apoptosis (Xu et al., 2001).

Apart from these evidences, no other studies have reported effects from *U. urealyticum* infection on sperm parameters. *U. urealyticum* infection had no effect on sperm function as observed during sperm analysis, in vitro bovine cervical mucus penetration assay and Hamster's oocyte sperm penetration assay (Shalhoub et al., 1986). In vitro, *U. urealyticum* experimental infection did not alter sperm motility or capability of penetration while spermatozoa were incubated with the germ for 45 min at very high *U. urealyticum*: spermatozoa ratios (up to 100 : 1) (Talkington et al., 1991). In vivo studies showed no statistically significant difference between sperm parameters in sub-fertile patients with or without *U. urealyticum* infection (Cintron et al., 1981) and no

correlation was found between abnormal sperm parameters and the presence of *U. urealyticum* in 86 unselected asymptomatic men (Gregoriou *et al.*, 1989). Similarly, infertile patients with *U. urealyticum* infection, diagnosed through PCR analysis of their semen sample, did not report any significant difference in seminal volume, sperm concentration, viability, motility, morphology and leucocyte number (Gdoura *et al.*, 2007). The same authors confirmed these findings in a group of asymptomatic male partners of infertile Tunisian couples who had the concomitant presence of *M. hominis* and *U. urealyticum* DNA in their semen samples (Gdoura *et al.*, 2008).

Mycoplasma hominis and other mycoplasmas

The effects of *M. hominis* on sperm parameters have often been evaluated together with other microorganisms (Hofstetter *et al.*, 1978; Bornman *et al.*, 1990; Corradi *et al.*, 1992; Gallegos *et al.*, 2008; Gdoura *et al.*, 2008). These studies showed a negative effect on sperm concentration (Gallegos *et al.*, 2008; Gdoura *et al.*, 2008), motility (Hofstetter *et al.*, 1978; Corradi *et al.*, 1992; Gallegos *et al.*, 2008) and morphology (Bornman *et al.*, 1990; Gallegos *et al.*, 2008; Gdoura *et al.*, 2008). Agbakoba *et al.* reported that many patients infected with various strains of mycoplasmas were oligozoospermics (Agbakoba *et al.*, 2007). The presence of *M. hominis* DNA in semen samples has been reported associated with low sperm concentration and abnormal sperm morphology; a negative correlation between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men has also been shown (Gdoura *et al.*, 2007).

A direct *in vitro* interaction between *M. hominis* and spermatozoa has also been analysed. An overnight incubation with various mycoplasma strains significantly decreased sperm motility and the percentage of normally shaped and acrosome-reacted spermatozoa (Rose & Scott, 1994). Ten minutes after incubation, *M. hominis* binds sperm heads, tails and the midpiece. Moreover, infected spermatozoa had the germ within the head and the midpiece cytosolic space. Only a subtle sperm damage was observed after a short-term *M. hominis* interaction with spermatozoa (Diaz-García *et al.*, 2006). Interestingly, based on experiment *M. genitalium* attaches to motile spermatozoa and therefore the microorganism may be carried with the female genital tract (Svenstrup *et al.*, 2003).

Spermatozoa pre-incubated with various strains of mycoplasmas showed lower penetration rate in Hamster oocytes compared with controls. A lower penetration rate has been reported in Percoll-washed spermatozoa which tested positive for the presence of mycoplasma DNA compared with those that showed no infection. The simi-

larities of hypo-osmotic swelling and kinematic parameters between the two groups suggest that the reduced sperm-oocyte penetration rate is not attributed to the latter two parameters (Kalugdan *et al.*, 1996).

By contrast, a number of studies failed to show any effect of mycoplasmas on sperm parameters both *in vivo* and *in vitro*. The presence of *M. hominis* and/or *U. urealyticum* in the semen was not associated with any significant difference in the sperm parameters of men attending an IVF unit (Hill *et al.*, 1987). Eggert-Kruse *et al.* reported no difference in conventional sperm parameters following the antimicrobial treatment of patients with *C. trachomatis*, *M. hominis*, *U. urealyticum* and *N. gonorrhoeae* infection (Eggert-Kruse *et al.*, 1988). Similar results were reported examining semen samples for routine analysis. Despite the high prevalence of mycoplasmas in these samples, conventional sperm parameters of infected men resulted similar to those of un-infected men (Andrade-Rocha, 2003). On this account, a systematic search for mycoplasmas infection has not been suggested (Rosemond *et al.*, 2006).

Candida albicans

Candida (C.) albicans infection alters sperm function. As a matter of fact, *C. albicans* experimental infection inhibits time-dependently sperm motility (Tuttle *et al.*, 1997). A significant inhibitory effect was only observed in samples with an initial yeast concentration of 20 mil/mL (Huwe *et al.*, 1998). A significant degree of sperm non-specific and a head-to-head (with *C. albicans* interposition) sperm agglutination was also found (Tian *et al.*, 2007). This led to the hypothesis that the formation of a mechanical barrier hampers sperm motility (Huwe *et al.*, 1998). However, mitochondrial and tail alterations which were later found in spermatozoa infected by *C. albicans* may contribute to the motility decline. In addition, spermatozoa in contact with *C. albicans* undergo acrosomal swelling, vesiculation (outer membrane) and rupture which may prejudice sperm fertilization capability (Tian *et al.*, 2007).

In this regard, we reported that the presence of *C. albicans* resulted in no fertilization after IVF and ICSI (Burrello *et al.*, 2004). We then showed that experimentally induced *C. albicans* co-incubation with spermatozoa isolated from normozoospermic healthy men significantly reduced sperm motility and increased the percentage of spermatozoa with low MMP or PS externalization (Burrello *et al.*, 2009). In this *in vitro* experimental model, *C. albicans* did not have any significant effect on sperm DNA fragmentation or chromatin integrity. On the other hand, the abnormal sperm chromatin compactness and DNA fragmentation found in a patient with

C. albicans infection (Burrello *et al.*, 2004) suggest that these effects of *C. albicans* require the presence of other factors (leucocyte, etc.) which are present in vivo.

More recently, it has been shown that farnesol, a sesquiterpene alcohol produced by many organisms which acts as a quorum sensing molecule and as a virulence factor of *C. albicans*, reduces sperm motility and causes sperm apoptosis and necrosis. Moreover, sub-lethal doses of this signalling molecule induce premature acrosome loss (Rennemeier *et al.*, 2009).

Trichomonas vaginalis

Trichomonas (*T.*) *vaginalis* is a flagellated parasite often found as an occult resident of the genital tract of sexually active women and men. Its presence in the seminal samples of asymptomatic men resulted in a significant increase of viscosity and number of particulate debris, decreased sperm motility, number of normal forms and viability. After a single course of treatment with metronidazole, a significant improvement of the semen characteristics was observed in about half of treated patients (Gopalkrishnan *et al.*, 1990). These findings suggest that *T. vaginalis* may cause infertility.

In vitro, this protozoan has been shown capable of reducing sperm motility without causing any sperm agglutination (Tuttle *et al.*, 1977). Following studies confirmed a detrimental effect of *T. vaginalis* on sperm motility and attempted to establish the mechanism(s) through which this takes place (Jarecki-Black *et al.*, 1988; Han *et al.*, 2004; Kranjčić-Zec *et al.*, 2004; Benchimol *et al.*, 2008). Jarecki-Black *et al.* reported that spent medium of *T. vaginalis* culture abolished sperm motility after 15 min of incubation. Trophozoite soluble fraction or formalin-killed trophozoites caused a 50% reduction in sperm motility, compared with the 25% reduction caused by the trophozoite particulate fraction or the sterile medium and 3% by saline (control). The *T. vaginalis* spermicidal activity was heat-stable, trypsin-sensitive and had a molecular weight of 12–15 kDa by gel filtration. This proteinaceous substance was present in and secreted by *T. vaginalis* trophozoites during normal growth in axenic culture (Jarecki-Black *et al.*, 1988). An inhibitory role of *T. vaginalis* metabolites (Han *et al.*, 2004) or of a soluble extract (Kranjčić-Zec *et al.*, 2004) of these protozoan on sperm motility was further reported. Incubation with a *T. vaginalis* soluble factor also resulted in increased viscosity, number of debris and in vitro sperm membrane damage (Kranjčić-Zec *et al.*, 2004). *T. vaginalis* binds also sperm head and flagella and that the reduction of sperm motility was associated with an intense agglutination. In this regard, *T. vaginalis* appears to be much more virulent than *T. foetus* whose effects were evaluated

in the same study on bull spermatozoa (Benchimol *et al.*, 2008).

By contrast, Daly *et al.* did not report any effect of *T. vaginalis* on sperm motility after up to 24 h of incubation, although the protozoa survived well in the semen samples (Daly *et al.*, 1989). The lack of effect may relate to low number of *T. vaginalis* (about 2500/mL semen) used in this study compared with the higher range (10^4 – 10^7 protozoa/mL) used in other studies (Tuttle *et al.*, 1977).

Effects of viruses

Hepatitis B and C viruses

Several studies have analysed the effects of *Hepatitis B virus* (HBV) or *Hepatitis C virus* (HCV) infection on sperm parameters. Sperm parameters of HCV-affected patients do not differ from those of non-infected ones (Garrido *et al.*, 2005). We evaluated the sperm parameters of infertile patients in Child-Pugh classification A with HBV or HCV infection, compared with those of a group of 30 patients with primary infertility because of other causes besides liver diseases. HBV patients (median HBV-DNA load of 6×10^5 copies/mL) had sperm density, total number, forward motility, morphology and viability significantly worse than those found in patients with HCV (median HCV-RNA load of 2.3×10^6 copies/mL). No significant correlation between sperm parameters and the duration of viral infection or the viral HBV-DNA load was found besides sperm morphology which exhibited a trend for a negative correlation with the viral HBV-DNA load (Vicari *et al.*, 2006a). HCV-infected patients had a significantly lower sperm motility and percentage of normal forms than controls. Combined antiviral treatment with interferon and ribavirin worsened sperm morphology but did not have any effect on the other sperm parameters (Durazzo *et al.*, 2006). A negative effect on sperm motility (Moretti *et al.*, 2008; Lorusso *et al.*, 2010) and morphology (Lorusso *et al.*, 2010) has been confirmed in HCV- and HBV-positive patients. However, Moretti *et al.* did not find any significant effect on sperm concentration (Moretti *et al.*, 2008), whereas Lorusso *et al.* found lower sperm concentration and viability in both HBV and HCV seropositive men compared with controls (Lorusso *et al.*, 2010).

Very little is known about the mechanism by which HBV affects sperm function. The HBV S protein (HBs), the main component of HBV envelop protein, reduces sperm motility in a dose- and time-dependent manner and increases the number of spermatozoa with low MMP. The fertilization rate in HBs-treated spermatozoa was significantly lower than that of controls (Zhou *et al.*, 2009).

Electronic microscopy revealed some significantly higher values of sperm apoptosis and necrosis in patients with HBV- or HCV-infection compared with controls, whereas the disomy and diploidy rates for chromosomes 18, X and Y did not significantly differ from controls (Moretti *et al.*, 2008). By contrast, significantly higher total sperm chromosome abnormalities, evaluated after zona-free Hamster oocyte penetration, were found in patients with HBV infection compared with those in healthy men. In addition, sperm chromosomes in HBV patients present stickiness, clumping, failure to stain, etc. These findings suggest that HBV infection may cause sperm chromosome aberrations (Huang *et al.*, 2003).

The possibility of HBV integrating into sperm chromosomes has been studied in patients with HBV infection. Specific fluorescent spots for HBV DNA have been spotted in sperm chromosomes, although with a different intensity. These results suggest the possibility of vertical transmission of HBV through the germ line to the next generation (Huang *et al.*, 2002, 2003).

Human immunodeficiency virus type 1

The effect of human immunodeficiency virus (HIV) type 1 infection on sperm parameters was evaluated in asymptomatic or minimally symptomatic HIV-seropositive men and in men with AIDS. All the men with AIDS had leucocytopenia and abnormal spermatozoa. By contrast, the sperm parameters of seropositive men did not significantly differ from those regarding healthy seronegative donors. Zidovudine therapy did not affect sperm morphology or seminal characteristics (Krieger *et al.*, 1991). No alteration in sperm parameters was later confirmed in HIV seropositive men (Dondero *et al.*, 1996). However, this study showed that HIV seropositive men had a significantly higher percentage of: (i) spermatozoa with cytoplasmic droplet, (ii) immature germ cells, and (iii) spermophages. In addition, HIV seropositive men showed a significant positive correlation between blood CD4+ and sperm motility as well as a significant inverse correlation between CD4+ and sperm abnormalities (Dondero *et al.*, 1996).

In contrast to seropositive men, those with HIV type 1 have a significantly lower ejaculate volume, sperm concentration, total count, progressive motility and normal morphology compared with controls. A significant positive correlation was observed between CD4 count and sperm concentration, total count, motility and progressive motility (Nicopoulos *et al.*, 2004). These data prove that sperm parameters are significantly impaired by the presence of HIV infection. Men with HIV have been reported to have low sperm motility compared with HIV negative ones and leucocytopenia irrespective of a previous

history of sexual transmitted diseases. These findings suggest that sperm motility impairment in HIV positive men may be related to an increase in oxidative stress leucocyte-mediated (Umaphy, 2005). However, Garrido *et al.* did not find any significant alteration in the sperm parameters of HIV-affected patients compared with non-infected ones (Garrido *et al.*, 2005). As a result of this inconsistency in the results of sperm parameters concerning HIV-infected men, Bujan *et al.* investigated sperm parameters in a large number of HIV type 1-infected patients and compared them with those belonging to a control group of fertile, non-infected men. They found that semen volume, percentages of progressive motile spermatozoa, total sperm counts and seminal leucocytes were lower, while pH values and spermatozoa multiple anomaly indices were higher in HIV-infected patients (Bujan *et al.*, 2007). Abnormal sperm parameters have been found in 83% of HIV-infected patients and in 42% of the HIV-uninfected male partners of HIV-infected women seeking fertility with an Odds ratio of 7 (95% CI = 2.1–23) (Coll *et al.*, 2007). Principal component analysis method showed that HIV-positive men have the worst sperm parameters, whereas the distribution of mannose receptors and cytokine levels in HIV-1-positive men were similar to those in uninfected individuals. The similar distribution of mannose receptors suggests that spermatozoa from infected individuals normally interact with oocytes (Cardona-Maya *et al.*, 2009). A recent study on HCV-HIV seropositive men showed that the only sperm parameter affected was progressive motility which was significantly lower than in controlled ones (Lorusso *et al.*, 2010). TUNEL analysis revealed an increased percentage of DNA-fragmented ejaculated spermatozoa in semen of HIV-infected men (Muciaccia *et al.*, 2007).

A prolonged exposure to asymptomatic, untreated HIV-1 infection does not seem to affect sperm parameters. As a matter of fact, no significant variation was observed in 55 men with HIV-1 infection whose sperm parameters were evaluated biannually for an average follow-up period of 77 weeks. These findings should be reassuring for untreated men infected with HIV-1 who wish to have children (van Leeuwen *et al.*, 2008a).

Apart from HIV, many drugs which are used for the treatment of HIV-infected men have profound spermotoxic effect. Nucleoside analogues reverse transcriptase inhibitors (NRTI), used for treating HIV-infected patients, have important adverse effects which are related to a common mechanism: alteration of mitochondrial activity. Given the relevant role played by these organelles on sperm function, the effects of these drugs have been evaluated on sperm function. Studies suggest that NRTI exposure alters mitochondrial energy-generating ability in spermatozoa. NRTI are known to increase ROS

production resulting in a decreased MMP. Reduced MMP leads to the release of some specific apoptotic factors, such as cytochrome C, which initiates programmed cell death (Sergerie *et al.*, 2004). The effects of antiretroviral therapy on semen quality were longitudinally evaluated in a cohort of male patients with a different estimated duration of HIV-1 infection. The average follow-up period was of 48 weeks. Five patients underwent thymidine analogue-containing treatment, 23 used tenofovir-based treatment and six used other regimens. At all time-points, the percentage of progressively motile spermatozoa was low and it significantly decreased from 28–17% during follow-up. All other semen parameters were in the normal range and remained stable (van Leeuwen *et al.*, 2008b).

Papillomavirus

Over the years, the role of papillomaviruses (HPV) on sperm parameters and/or function has been examined with contrasting results. The presence of HPV gene sequences have been shown in the 64% of Percoll-separated spermatozoa. The HPV type 16 was detected about twice more frequently than the type 18 (Chan *et al.*, 1994). Lay *et al.* not only reported that HPV types 16 and 18 are able to infect human spermatozoa, but that some of their genes are actively transcribed in the infected germ cells (Lai *et al.*, 1996). Following experimental infection, the viral DNA appears tenaciously bound to spermatozoa suggesting an internalization into spermatozoa. As a matter of fact, sperm washing (centrifuge, two-layer Isolate colloid wash or test-yolk buffer procedures) was not able to remove exogenous HPV DNA (Brossfield *et al.*, 1999).

In the attempt to clarify the mechanism(s) by which HPV binds to spermatozoa, Pèrez-Andino *et al.* reported that the capsids of HPV type 16 specifically interact with spermatozoa. Purified HPV16 virions directly absorb to alive spermatozoa in native semen and in conditions resembling the female genital tract. In particular, the authors found that HPV16 capsids bind to two distinct sites at the equatorial region of the sperm head surface (Pèrez-Andino *et al.*, 2009). More recently, the presence of HPV DNA has been shown in about 25% of the sperm heads in infected teenagers (18-years old) who had an unprotected sexual intercourse. However, the authors could not explain whether the virus was integrated in the nucleus or not (Foresta *et al.*, 2010a). The presence of the virus creates spermatozoa carriers for the sexual transmission of HPV to sexual partners.

In vivo, HPV infection seems to inhibit V in sperm motility. As a matter of fact, the prevalence of asthenozoospermia is higher in HPV (type 16 and 18)-positive patients compared with patients without infection. Nevertheless, many sperm kinematic parameters did not differ

significantly between the two groups (Lai *et al.*, 1997). A reduction of sperm motility has been recently shown in infertile patients and individuals with risk factors, in particular when the infection was present in spermatozoa (Foresta *et al.*, 2010b), and in teenagers (Foresta *et al.*, 2010a). By contrast, no effects on semen quality and assisted reproductive technique (ART) variables (pregnancy and abortion rates) have been reported in men and women who were positive for HPV type 16 (Tanaka *et al.*, 2000). The lack of effect on the HPV infection on sperm parameters has also been confirmed by Rintala *et al.* As a matter of fact, the presence of HPV DNA did not affect semen volume, sperm concentration, motility and vitality. Neither oligo- nor asthenozoospermia was associated with the presence of seminal HPV DNA (Rintala *et al.*, 2004).

Using an experimental in vitro model of infection, HPV DNA seems to increase sperm motility. HPV DNA increased sperm motility total and progression, evaluated by CASA. This suggests that HPV DNA increases sperm metabolism or enhances the calcium-regulated motility mechanism. Although an artefact of PCR products cannot be ruled out (Brossfield *et al.*, 1999), Connelly *et al.* confirmed that normal spermatozoa had higher motility after incubation with HPV types 16, 18, 31 and 33, but not 6/11, and increased linearity after being incubated with all HPV types tested with the exception of the type 18 (Connelly *et al.*, 2001). An opposite effect of HPV types 6b/11, 16, 18, 31 and 33 exposure has been reported on motility (decreased) and hyperactivation (increased) which suggests that HPV-exposed spermatozoa retain some fertilizing capacity (Lee *et al.*, 2002).

Normal motile spermatozoa incubated with E6-E7 HPV DNA fragments had increased DNA fragmentation after exposure to the DNA of HPV types 16 and 31, whereas types 18, 33 and 6/11 did not alter sperm DNA integrity (Connelly *et al.*, 2001). While attempting to evaluate any further role of HPV on sperm DNA of specific gene regions, Lee *et al.* examined the effects of HPV exposure to the integrity of exons 5 and 8 of the p53 gene. Fragmentation of exon 5 occurred after exposure to HPV DNA type 18. By contrast, only exon 8 was affected by HPV type 16. HPV DNA from type 31 or 33 was without effect on the p53 exons (Lee *et al.*, 2002).

Effects of the oxidative stress

An increased production of ROS and/or a decrease of the antioxidant defences cause sperm abnormalities. These include decreased sperm motility, acrosine activity and sperm-oocyte fusion capability (see Lanzafame *et al.*, 2009; for review). As a matter of fact, a <25% sperm-oocyte penetration rate is associated with an increased ROS production in an elevated number of oligozoospermic

patients with abnormal sperm function (Aitken *et al.*, 1989). Sperm motility inhibition caused by ROS has been reported to correlate negatively with MDA seminal plasma levels (Saraniya *et al.*, 2008), whereas a decrease of MDA is associated with an increased pregnancy rate (Suleiman *et al.*, 1996). An increased oxidative stress was suggested to cause seminal plasma hyperviscosity in infertile males (Aydemir *et al.*, 2008).

An increased oxidative stress damages also sperm chromatin/DNA integrity. As a matter of fact, exposure to ROS increases DNA fragmentation in normal spermatozoa (Aitken *et al.*, 1998); causes DNA protein cross-linking in chromatin (Twigg *et al.*, 1998); increases the frequency of DNA single and double strand breaks (Barroso *et al.*, 2000); and oxidates DNA base changes in asthenozoospermic and normozoospermic infertile patients compared with fertile men (Kodama *et al.*, 1997). Sperm DNA fragmentation does not correlate with the fertilization rate, but is associated with a significant reduction of pregnancy rate in ART programmes when TUNEL-positive spermatozoa are used (Henkel *et al.*, 2003). Therefore, spermatozoa with damaged DNA are able to fertilize oocytes, but at the time when the paternal genome is switched on, any further development is stopped (Evenson *et al.*, 2002). DNA damage seems to lead to an amplified risk of miscarriage and chromosomal abnormalities (Griveau & Le Lannou, 1997).

Effects of pro-inflammatory cytokines

Cytokines are soluble mediators produced by lymphoid and non-lymphoid cells that play a key role in the afferent and efferent phases of both innate and acquired immune responses. In the dynamic of the inflammatory response, cytokines have pleiotropic and redundant effects. For example, tumour necrosis factor- α (TNF α) is present in the initial inflammatory trigger, but it is also an inductor of chemokins, participates in the neutrophil chemotaxis, enhances the toxic final effect and induces apoptosis; interleukin-6 (IL-6) participates in the initial inflammatory trigger, but it also causes activation and differentiation of leucocytes and participates to the final toxic effect through ROS overproduction; interleukin-8 (IL-8) participates to the neutrophil chemo-attraction phase in the inflamed site and neutrophil activation towards phagocytosis. Thus, cytokines have a multitasking role which may disturb male accessory gland function.

Interleukin 1

Interleukin 1 (IL-1) concentration in the seminal plasma has been reported to be higher in infertile patients than in normal controls. However, no difference was found in

different subgroups of patients divided on the basis of progressive motility or percentage of sperm with abnormal forms (Dousset *et al.*, 1997). IL-1 does not have any effect on both spontaneous or calcium ionophore-induced acrosome reaction in normal spermatozoa (Dimitrov & Petrovská, 1996) as well as on sperm MDA production in vitro when used alone or in combination with leucocytes (Fraczek *et al.*, 2008).

Interleukin 6

Seminal plasma IL-6 concentration is higher in infertile patients than in normal fertile men. It also correlates negatively with sperm MDA, suggesting an ROS-mediated lipoperoxidation process (Camejo *et al.*, 2001). An inhibitory dose- and time-dependent effect of IL-6 on sperm motility was reported in vitro and seems to relate to overproduction of nitric oxide (NO) (Lampiao & du Plessis, 2008). In addition, IL-6 can inhibit both spontaneous and calcium ionophore- or progesterone-induced acrosome reaction of normal spermatozoa. However, this inhibitory effect had a lower intensity compared with the one obtained when spermatozoa are incubated with TNF α in the same experimental model (Lampiao & du Plessis, 2009).

Interleukin 8

IL-8 has no effect on sperm motility and on the ionophore-induced acrosome reaction in vitro (Fedder & Ellerman-Eriksen, 1995). By contrast, seminal plasma IL-8 concentrations negatively correlate with the total number of motile spermatozoa or with the number of motile spermatozoa harvested after swim-up technique in subfertile patients. A significant positive correlation was found between seminal plasma IL-8 and leucocyte counts (Eggert-Kruse *et al.*, 2001). An increasing effect of IL-8 has also been reported on normal spermatozoa in vitro, both after physiological or infection-inflammation concentrations (Martínez *et al.*, 2007).

Interferon- γ

A significant inhibitory effect of interferon- γ (IFN γ) on sperm motility was reported in vitro (Hill *et al.*, 1987; Fedder & Ellerman-Eriksen, 1995). Such an effect was confirmed in experiments using both TNF α and IFN γ (Estrada *et al.*, 1997). However, a subsequent study did not replicate this finding (Sikka *et al.*, 2001). Sperm motility inhibition was associated with a significantly reduced capacity of spermatozoa to penetrate Hamster oocytes (Hill *et al.*, 1989). At physiological concentration, IFN γ increased sperm membrane lipoperoxidation, but

no further increment of MDA production was observed when this cytokine was used at higher concentrations, such as those found in the course of infection/inflammation (Martínez *et al.*, 2007). IFN γ has no significant effect on calcium ionophore-induced acrosome reaction (Fedder & Ellerman-Eriksen, 1995), whereas it has a suppressive effect on spontaneous acrosome reaction and acrosome activity (Bian *et al.*, 2007). A marked reduction of Na⁺/K⁺-ATPase, Ca⁺⁺-ATPase and super-oxide dismutase activities and an increased production of NO have been observed in normal spermatozoa incubated with IFN γ (Bian *et al.*, 2007). These latter effects may explain the detrimental effects of IFN γ on sperm acrosome activity and acrosome reaction.

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, is a constituent of the seminal plasma. It is expressed in the epididymis and it is an important factor of sperm maturation (Eickhoff *et al.*, 2004). Sperm-associated, but not seminal plasma, MIF negatively correlates with sperm motility (Frenette *et al.*, 2005). We have shown a negative correlation between MIF levels in human seminal fluid and fertility status. In addition, MIF added to normal spermatozoa decreased total sperm and progressive motility and increased the percentage of spermatozoa with PS externalization or with DNA fragmentation (Aljabari *et al.*, 2007). A deleterious effect on sperm motility was also reported although only at high concentrations, whereas MIF may play a physiological role in sperm capacitation process at lower concentrations (Carli *et al.*, 2007).

Tumour necrosis factor- α

Hussanet *et al.* reported that TNF α is present in the seminal plasma of normal men at a concentration similar to that found in the seminal plasma of patients with bacterial infection (Hussanet *et al.*, 1993). On the other hand, other studies have shown that seminal plasma TNF α concentrations are higher in patients with bacterial or mycoplasma infections than in those found in men without infection (Gruschwitz *et al.*, 1996). In addition, it has been shown that leucocytospermia (Omu *et al.*, 1999; Sikorski *et al.*, 2001) and/or bacteriospermia (Omu *et al.*, 1999) are associated with a higher release of TNF α .

Although several studies have analysed the effect of TNF α on sperm parameters, no clear conclusion can be drawn. Sperm motility (Wincek *et al.*, 1991; Fedder & Ellerman-Eriksen, 1995), Hamster oocyte penetration (Wincek *et al.*, 1991) and ionophore-induced acrosome reaction (Fedder & Ellerman-Eriksen, 1995) are not affected by the incubation with TNF α . Haney *et al.* reported that motile spermatozoa obtained from fertile

men and separated by the swim-up technique did not show any decreased motility following exposure to TNF α , IL-1 α and IFN γ alone or even in combination with higher doses than those observed in vivo (Haney *et al.*, 1992). Accordingly, no relationship between seminal plasma TNF α concentration and sperm parameters were reported in normal men (Hussanet *et al.*, 1993). No effects of TNF α on sperm viability have been reported (Lewis *et al.*, 1996).

On the other hand, significant in vitro negative effects of TNF α on sperm motility and sperm fertilizing ability of Hamster oocytes have been reported (Hill *et al.*, 1987, 1989). Similarly, Gruschwitz *et al.* showed that seminal plasma TNF α concentrations in patients with bacterial or mycoplasma infections correlated negatively with the number of progressively motile spermatozoa (Gruschwitz *et al.*, 1996). Kocak *et al.* reported that TNF α levels correlate negatively with sperm motility and morphology, but not with total sperm counts (Kocak *et al.*, 2002). Estrada *et al.* showed that although the inflammatory cytokines TNF α plus IFN γ only have partial detrimental effects on sperm motility, viability, membrane integrity and lateral head displacement, they may contribute to the poor fertilizing potential of human spermatozoa during inflammatory conditions (Estrada *et al.*, 1997). Accordingly, the peritoneal fluid in women with endometriosis containing elevated concentrations of TNF α caused a significant reduction in both total and progressive sperm motility compared to spermatozoa incubated with peritoneal fluid which did contain TNF α . The ability of TNF α to hamper sperm motility in vitro suggests that this may be a mechanism for the infertility observed in women with minimal endometriosis (Eisermann *et al.*, 1989). We found that TNF α inhibits total and progressive sperm motility in a concentration- and time-dependent manner (Perdichizzi *et al.*, 2007). This detrimental effect may relate to a reduced sperm mitochondrial function, as shown by an increased number of spermatozoa with low MMP (Bian *et al.*, 2004; Perdichizzi *et al.*, 2007) as well as to an increased NO production (Lampiao & du Plessis, 2008).

Divergent results have been reported concerning the effects of TNF α on lipid sperm membrane peroxidation, evaluated by the production of malondialdehyde. As a matter of fact, TNF α has been reported to increase both MDA production at physiological concentrations and, to a greater extent, at infection-inflammation concentrations (Martínez *et al.*, 2007) and to have no effect on MDA production from spermatozoa isolated by swim-up technique (Fraczek *et al.*, 2008).

In contrast with the findings by Fedder & Ellerman-Eriksen (1995), TNF α has been reported to inhibit spontaneous and induced (by calcium ionophore or progesterone) acrosome reaction in normal spermatozoa

(Dimitrov & Petrovská, 1996; Bian *et al.*, 2007; Lampiao & du Plessis, 2009).

Together with the previous observation showing TNF α capable of inducing apoptosis, we found that this pro-inflammatory cytokine also causes sperm apoptosis. As a matter of fact, TNF α increased both the percentage of PS externalization, an early molecular event of apoptosis and DNA fragmentation, a late sign of apoptosis. Similar TNF α toxic effects were reported on sperm motility, functional integrity of the sperm membrane and DNA fragmentation. These effects were reversed by co-incubation with infliximab, a selective TNF- α antibody (Said *et al.*, 2005). A positive correlation has recently been reported between seminal plasma TNF α levels and apoptotic spermatozoa as shown by an increased percentage of spermatozoa with PS externalization (Allam *et al.*, 2008).

Epididymal macrophages and dendritic cells

In normal conditions, the presence of immune cells, including lymphocytes, macrophages, mast cells and DCs have been described in the epididymis and the testis of different species (Wang & Holstein, 1983; Yeung *et al.*, 1994; Hooper *et al.*, 1995; Hedger & Meinhardt, 2003).

Macrophages and DCs make up the main population of antigen presenting cells (APC) within the inflamed rat testis; they play an important role in the initiation and maintenance of autoimmunity (Rival *et al.*, 2007) as shown by flow cytometric analysis (Suescun *et al.*, 2003) and immunohistochemical techniques (Rival *et al.*, 2006). The large population of resident epididymal and testicular macrophages, as well as of DCs in the testis, is strongly involved in mediating this specialized immunological environment. Testicular macrophages have been described as cells with an immune-suppressor profile, thus contributing to the immune-privilege of the testis (Naito & Itoh, 2008).

Immune responses within the testis are regulated in a manner that provides protection for the developing male germ cells, while permitting qualitatively normal inflammatory responses and protection against infection (Naito & Itoh, 2008). Two primary mechanisms prevent autoimmune reaction initiation against spermatozoa: anatomical sequestration by blood-testis and blood-epididymis barriers and active immune-suppression by seminal plasma components and suppressor/cytotoxic lymphocytes (CTLs) (Pöllänen & Cooper, 1994).

Several studies have characterized both the phenotype and the distribution of immune cells within the testis and in different epididymal regions of normal and infertile men. The testis has a unique immune structure which helps protect spermatogenesis from the recognition of the immune system (Comphaire *et al.*, 1999). This immune-

testicular barrier may explain the higher testicular CD8_{pos}/CD4_{neg} ratio compared with the general circulation (Witkin *et al.*, 1996). Phagocytic macrophages as well as APCs (Comphaire *et al.*, 1999) have a cross-talk effect with Leydig cells that may play a role in normal spermatogenesis. Therefore, a balance between the immune structures is important for keeping a suitable microenvironment for normal spermatogenesis.

Naito & Itoh (2008) reported that the tubuli recti (TR) in the testis have a specific region which attracts lymphocytes. Many antigen-presenting macrophages preferentially accumulate around the TR in normal conditions. This characteristic macrophage accumulation is an acquired phenomenon that is completed when spermatids begin to differentiate within the seminiferous tubules (Fijak & Meinhardt, 2006). Furthermore, intra-tubular lymphocytes that are very close to both germ cells and their remnants could be occasionally found in the TR, rete testis and epididymis, but not in the seminiferous tubules of normal animals (Itoh *et al.*, 2005). Most intra-epithelial lymphocytes (IELs) of the rete testis, epididymis and vas deferens positively stain for T-lymphocyte markers and a major proportion of epididymal IELs express the CD8 suppressor/cytotoxic phenotype (Fijak & Meinhardt, 2006). Although the physiological function of these penetrating lymphocytes remains unknown, it is supposed that this micro-status provides the opportunity for immune reaction in some pathological conditions (Naito & Itoh, 2008).

Epididymal macrophages

An infection often leads to an acute inflammatory response which activates resident macrophages and gathers blood leucocytes towards the site of inflammation (Hedger & Meinhardt, 2003). The major macrophage activities, including phagocytosis and production of either inflammatory or anti-inflammatory mediators, are controlled by surface receptors. In rats, intracytoplasmic antigen ED1 is expressed in monocytes, subpopulations of newly arrived tissue macrophages and DCs (Dijkstra *et al.*, 1985; Bañuls *et al.*, 1993), whereas the ED2 surface glycoprotein is selectively expressed in resident macrophages (Polfliet *et al.*, 2006). ED1- and a heterogeneous population of ED2-positive macrophages have been characterized in rat testes (Gerdprasert *et al.*, 2002; Hedger, 2002; Hedger & Meinhardt, 2003; O'Bryan *et al.*, 2005). Lipopolysaccharide-induced inflammation in this tissue results in a transient and significant increase of ED1-positive macrophages, which express inflammatory markers even in the absence of exogenous inflammatory stimulation, concomitantly with no change in the population of resident ED2 macrophages (Gerdprasert *et al.*, 2002). The

functional role of the heterogeneous population of ED2-positive macrophages as well as other immune epididymal cells and their impact in orchestrating the link between innate and adaptive immune response to an infectious stimulus in the epididymis, however, is to be defined and requires further investigation (Rodrigues *et al.*, 2008).

Testicular dendritic cells

Dendritic cells play an important role in the initiation and maintenance of autoimmunity (Rival *et al.*, 2007). These are considered the pacemakers of the immune response as they act as a bridge between the innate and adaptive immune systems. The recognition of a 'danger' signal initiates the maturation of DCs which ultimately activate cells of the adaptive arm of the immune system, B and T cells.

Dendritic cells are bone marrow-derived APCs found in most tissues of the body (Sigal & Rock, 2000). They are initially in an immature state which shows a high phagocytic activity and low levels of MHC class II and the co-stimulatory ligands CD80 and CD86 (Banchereau & Steinman, 1998; Guermonprez *et al.*, 2002). Once DCs acquire antigens and receive activation signals, for example, from CD40-CD40L interactions, Toll-like receptors and/or exposure to inflammatory cytokines, they mature and show high levels of MHC class II molecules such as CD80, CD86 and cytokines such as IL-12 (Banchereau & Steinman, 1998; Guermonprez *et al.*, 2002). They also migrate towards the T-cell zones of the secondary lymphoid organs where they interact with naive T cells. It is nowadays believed that DCs are the only cells which can stimulate naive T cells in vivo (Banchereau & Steinman, 1998; Mellman & Steinman, 2001). It has been recently shown that monocyte-derived DCs are recruited from blood monocytes into lymph nodes by lipopolysaccharide and live or dead Gram-negative bacteria (Cheong *et al.*, 2010).

Therefore, the phenotype of DCs plays an important role in the initiation of the immune response. Immature DCs are believed to induce tolerance to self antigens, whereas mature DCs promote immunity to foreign and self-antigens (Steinman, 1991). As a matter of fact, flow cytometric data, the lack of chemokine CCR7 and proinflammatory cytokine production show that DCs of the normal testis are not mature (semi-mature or immature). The semi-maturation of DCs represents a unique developmental tolerogenic stage for DCs and, without further stimulation, it is not necessarily 'dangerous' for the immune system of the testis. Cells appear comparable with steady-state migratory veiled DCs within the lymphatics, which seem to continuously tolerize T-cells in lymph nodes against tissue-derived self-antigens or apop-

otic cells (Stubbs *et al.*, 2001; Lutz & Schuler, 2002). On the other hand, during the development of chronic testicular inflammation, the DC population acquires the capacity to move to lymph nodes as indicated by high expression of CCR7, thereby stimulating antigen specific T-cell responses (Sainio-Pöllänen *et al.*, 1996). Furthermore, the numbers of DCs in the testicular interstitium strongly correlate with the development of inflammatory lesions in experimental autoimmune orchitis (EAO) which may be regarded as a model of organ-specific autoimmunity and of testicular inflammation, characterized by an interstitial inflammatory mononuclear cell infiltration, seminiferous tubule damage and germ cell apoptosis and infertility (Rival *et al.*, 2007).

Post-infective secretory alterations

During the infection of the male genital tract, microorganisms lead to a defence reaction in the accessory gland(s) site in which invasion is taking place, for example unspecific and specific immune reactions, with final transfer of oxidative damage from the infected tissues to the spermatozoa located in the post-testicular sperm reserves.

Secretory gland dysfunction, triggered by microbial noxae and/or inflammatory reaction, is the most important cause of negative impact on sperm quality. It is expressed through a non-specific chronic inflammatory reaction [leucocytospermia, increased seminal plasma pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF α , etc.), ROS overproduction and/or specific autoimmune response (overproduction of antisperm autoantibodies)] (Comphaire *et al.*, 1999; Ochsendorf, 1999). These bioactive substances may continue to be found after an apparent post-antibiotic microbial eradication because of the continuation of oxidative stress and gradual consumption of the scavenger systems during the inflammatory response, particularly micronutrients provided by the epididymal plasma.

Tissue damage caused by infection/inflammation can impair the secretory function of male accessory glands (epididymis, prostate and seminal vesicles). Secretory damage of the prostate during inflammatory prostatitis (category IIIA according to the NIH classification) is shown by an increase of granulocytes in prostatic secretions included in the NIH diagnostic criteria (Ludwig *et al.*, 2002).

A functional deficiency of one or more glands can be identified by measuring the secretory products of these glands in the seminal plasma. Many of these products, such as α -glucosidase, fructose, prostaglandins, zinc and citric acid among others, are of crucial importance in sperm physiology. Inflammation 'per se' (Mahmoud

et al., 1998) and secondary obstruction (Dohle, 2003) have been proposed as possible mechanisms through which different infectious agents may impair male accessory gland function.

Under normal conditions, epididymal secretory factors are involved in the maturation of spermatozoa and its function can be evaluated by measuring L-carnitine, glycerylphosphoryl choline and α -glucosidase in the seminal plasma. The secretion of α -glucosidase is used to evaluate epididymal function in an accurate way; however, there is no consensus regarding the impact of chronic epididymitis on the level of this marker (Cooper *et al.*, 1990; Wolff *et al.*, 1991; Mahmoud *et al.*, 1998). The seminal vesicles produce fructose, ascorbic acid, ergothioneine, prostaglandins and bicarbonate. These factors act as reducing agents and prevent sperm agglutination (Okamura *et al.*, 1986). A deleterious effect of infection on the secretory function of the seminal vesicles, evaluated by fructose levels, has been previously reported (Comhaire *et al.*, 1989); however, these findings have not been confirmed yet by other authors (Cooper *et al.*, 1990; Bezold *et al.*, 2007). The secretory function of the prostate gland has been widely investigated: seminal plasma pH, citric acid, γ -Glutamyl transpeptidase and zinc have been proposed as markers for its exocrine function and seminal plasma concentrations are usually altered during bacterial infection and inflammation (Weidner *et al.*, 1999). However, they are currently not recommended as diagnostic tools for detecting inflammation or infection (Ludwig *et al.*, 2002).

Marconi *et al.* (2009) found that the levels of α -glucosidase, fructose, zinc and sperm concentration were significantly lower in patients with infection compared with the control group, although there were no conclusive findings that indicated a total obstruction of the male reproductive tract at any level. This fact confirms that obstruction is not an important cause of impairment of the male accessory gland function during infection. Although infection has been previously mentioned as common cause of male reproductive tract obstruction (World Health Organization, 2000), our findings and more recent studies (Ludwig *et al.*, 2003; Weidner *et al.*, 2008) seem to confirm that it occurs rarely in patients with male reproductive tract infection and inflammation. Macroscopic analysis of the ejaculate has also been reported as clinically useful method to evaluate the secretory activity of the seminal vesicles and prostate. Abnormal coagulation, liquefaction, volume, viscosity and pH strongly cause gland dysfunction.

Conclusion

Although there is an ongoing open debate with pros and cons on the role of MAGI concerning male infertility, many

evidences suggest that MAGI should be considered as a risk factor for male infertility (Bayasgalan *et al.*, 2004). As matter of fact, MAGI may impair sperm function and cause male infertility through the multiple patho-physiological mechanisms which have previously been shown.

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