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REVIEW ARTICLE

Male accessory gland infection and sperm parameters (review)

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Summary

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 transrectal 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	*History:* positive for urinary infection, epididymitis and/or sexually transmitted disease *Physical signs:* thickened or tender epididymis, tender vas deferens and/or abnormal digital rectal examination
B	*Prostatic fluid:* abnormal prostate fluid expression and/or abnormal urine after prostatic massage
C	*Ejaculate signs:* 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.*) *urealitycum* 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; Bayasgalan *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 doxycyclin, 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 (Dìaz-Garcìa *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 similarities 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 lead 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; Kranjcić-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 (Kranjcić-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 (Kranjcić-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 leucocytospemia 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) spermiophages. 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 (Nicopoullos *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 leucocytospermia 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 (Umapathy, 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 papillomavirures (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 (TNFα) 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 TNFα 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 (IFNγ) on sperm motility was reported in vitro (Hill *et al.*, 1987; Fedder & Ellerman-Eriksen, 1995). Such an effect was confirmed in experiments using both TNFα and IFNγ (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, IFNγ 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). IFNγ 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 acrosine 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 IFNγ (Bian *et al.*, 2007). These latter effects may explain the detrimental effects of IFNγ on sperm acrosine 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 TNFα 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 (Hussenet *et al.*, 1993). On the other hand, other studies have shown that seminal plasma TNFα 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 TNFα.

Although several studies have analysed the effect of TNFα 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 TNFα. 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 TNFα, IL-1α and IFNγ alone or even in combination with higher doses than those observed in vivo (Haney *et al.*, 1992). Accordingly, no relationship between seminal plasma TNFα concentration and sperm parameters were reported in normal men (Hussenet *et al.*, 1993). No effects of TNFα on sperm viability have been reported (Lewis *et al.*, 1996).

On the other hand, significant in vitro negative effects of TNFα 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 TNFα 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 TNFα 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 TNFα plus IFNγ 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 TNFα caused a significant reduction in both total and progressive sperm motility compared to spermatozoa incubated with peritoneal fluid which did contain TNFα. The ability of TNFα 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 TNFα 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 TNFα on lipid sperm membrane peroxidation, evaluated by the production of malondialdehyde. As a matter of fact, TNFα 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), TNFα 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 TNFα capable of inducing apoptosis, we found that this pro-inflammatory cytokine also causes sperm apoptosis. As a matter of fact, TNFα increased both the percentage of PS externalization, an early molecular event of apoptosis and DNA fragmentation, a late sign of apoptosis. Similar TNFα 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 TNFα 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 heterogenous 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 dendritc 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 apoptotic 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 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, TNFα, 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.

References

Agarwal A, Saleh RA & Bedaiwy MA. (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 79, 829–843.

Agbakoba NR, Adetosoye AI & Ikechebelu JI. (2007) Genital mycoplasmas in semen samples of males attending a tertiary care hospital in Nigeria: any role in sperm count reduction? *Niger J Clin Pract* 10, 169–173.

Aitken RJ, Clarkson JS, Hargreave TB, Irvine DS, Wu FC. (1989) Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia. *J Androl* 10, 214–220.

Aitken RJ, Gordon E, Harkiss D *et al.* (1998) Negative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod* 59, 1037–1046.

Aljabari B, Calogero AE, Perdichizzi A *et al.* (2007) Imbalance in seminal fluid MIF indicates male infertility. *Mol Med* 13, 199–202.

Allam JP, Fronhoffs F, Fathy A *et al.* (2008) High percentage of apoptotic spermatozoa in ejaculates from men with chronic genital tract inflammation. *Andrologia* 40, 329–334.

Andrade-Rocha FT. (2003) *Ureaplasma urealyticum* and *Mycoplasma hominis* in men attending for routine semen analysis. Prevalence, incidence by age and clinical settings, influence on sperm characteristics, relationship with the leukocyte count and clinical value. *Urol Int* 71, 377–381.

Aydemir B, Onaran I, Kiziler AR *et al.* (2008) The influence of oxidative damage on viscosity of seminal fluid in infertile men. *J Androl* 29, 41–46.

Banchereau J & Steinman RM. (1998) Dendritic cells and the control of immunity. *Nature* 392, 245–252.

Bañuls MP, Alvarez A, Ferrero I, Zapata A & Aldavin C (1993) Cell-surface marker analysis of rat thymic dendritic cells. *Immunology* 79, 298–304.

Barroso G, Morshedi M & Oehringer S. (2000) Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. *Hum Reprod* 15, 1338–1344.

Bayasgalan G, Naranbat D, Radnaabazar J *et al.* (2004) Male infertility: risk factors in Mongolian men. *Asian J Androl* 6, 305–311.

Benchimol M, de Andrade Rosa I, da Silva Fontes R *et al.* (2008) Trichomonas adhere and phagocytose sperm cells: adhesion seems to be a prominent stage during interaction. *Parasitol Res* 102, 597–604.

Bezold G, Politch JA, Kiviat NB, Kuypers JM, Wolff H & Anderson DJ. (2007) Prevalence of sexually transmissible pathogens in semen from asymptomatic male infertility patients with and without leukocytospermia. *Fertil Steril* 87, 1087–1097.

Bian J, Guo X, Xiong C *et al.* (2004) Experimental study of the effect of rhTNF-alpha on human sperm mitochondrial function and motility in vitro. *Zhonghua Nan Ke Xue* 10, 415–419.

Bian SL, Jin HB, Wang SZ *et al.* (2007) Effects of interferon-gamma and tumor necrosis factor-alpha on the fertilizing capacity of human sperm and their mechanisms. *Zhonghua Nan Ke Xue* 13, 681–684.

Binnicker MJ, Williams RD & Apicella MA. (2003) Infection of human urethral epithelium with *Neisseria gonorrhoeae* factors and protects cells from staurosporine-induced apoptosis. *Cell Microbiol* 5, 549–560.

Bornman MS, Mahomed MF, Boomker D *et al.* (1990) Microbial flora in semen of infertile African men at Garankuwa hospital. *Andrologia* 22, 118–121.

Brossfield JE, Chan PJ, Patton WC *et al.* (1999) Tenacity of exogenous human papillomavirus DNA in sperm washing. *Int J STD AIDS* 15, 740–743.

Bujan L, Sergerie M, Moinard N *et al.* (2007) Decreased semen volume and spermatozoa motility in HIV-1-infected patients under antiretroviral treatment. *J Androl* 28, 444–452.

Burrello N, Calogero AE, Perdichizzi A *et al.* (2004) Inhibition of oocyte fertilization by assisted reproductive techniques and increased sperm DNA fragmentation in the presence of *Candida albicans*: a case report. *Reprod Biomed Online* 8, 569–573.

Burrello N, Salmeri M, Perdichizzi A *et al.* (2009) *Candida albicans* experimental infection: effects on human sperm motility, mitochondrial membrane potential and apoptosis. *Reprod Biomed Online* 18, 496–501.

Busolo F & Zanchetta R. (1984) Do mycoplasmas inhibit the human sperm fertilizing ability in vitro? *Isr J Med Sci* 20, 902–904.

Camejo MI, Segnini A & Proverbio F. (2001) Interleukin-6 (IL-6) in seminal plasma of infertile men, and lipid peroxidation of their sperm. *Arch Androl* 47, 97–101.

Cardona-Maya W, Velilla P, Montoya CJ *et al.* (2009) Presence of HIV-1 DNA in spermatozoa from HIV-positive patients: changes in the semen parameters. *Curr HIV Res* 7, 418–424.

Carli C, Leclerc P, Metz CN *et al.* (2007) Direct effect of macrophage migration inhibitory factor on sperm function: possible involvement in endometriosis-associated infertility. *Fertil Steril* 88(Suppl. 4), 1240–1247.

Chan PJ, Su BC, Kalugdan T *et al.* (1994) Human papillomavirus gene sequences in washed human sperm deoxyribonucleic acid. *Fertil Steril* 61, 982–985.

Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP *et al.* (2010) Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* 143, 416–429.

Cintron RD, Wortham JW Jr *et al.* (1981) The association of semen factors with the recovery of *Ureaplasma urealyticum*. *Fertil Steril* 36, 648–652.

Coll O, Lopez M, Vidal R *et al.* (2007) Fertility assessment in non-infertile HIV-infected women and their partners. *Reprod Biomed Online* 14, 488–494.

Comhaire F, Verschraegen G & Vermeulen L. (1980) Diagnosis of accessory gland infection and its possible role in male infertility. *Int J Androl* 3, 32–45.

Comhaire FH, Vermeulen L & Pieters O. (1989) Study of the accuracy of physical and biochemical markers in semen to detect infectious dysfunction of the accessory sex glands. *J Androl* 10, 50–53.

Comphaire FH, Mahmoud AM, Depuydt CE, Zalata AA & Christophe AB. (1999) Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: The andrologist's viewpoint. *Hum Reprod Update* 5, 393–398.

Connelly DA, Chan PJ, Patton WC *et al.* (2001) Human sperm deoxyribonucleic acid fragmentation by specific types of papillomavirus. *J Assist Reprod Genet* 184, 1068–1070.

Cooper TG, Weidner W & Nieschlag E. (1990) The influence of inflammation of the human male genital tract on secretion of the seminal markers alpha-glucosidase, glycerophosphocholine, carnitine, fructose and citric acid. *Int J Androl* 13, 329–336.

Corradi G, Molnàr G & Pànovics J. (1992) Andrologic significance of genital mycoplasma. *Orv Hetil* 133, 3085–3088.

Daly JJ, Sherman JK, Green L *et al.* (1989) Survival of *Trichomonas vaginalis* in human semen. *Genitourin Med* 65, 106–108.

Dìaz-Garcìa FJ, Herrera-Mendoza AP, Giono-Cerezo S *et al.* (2006) *Mycoplasma hominis* attaches to and locates intracellularly in human spermatozoa. *Hum Reprod* 21, 1591–1598.

Diemer T, Weidner W, Michelmann HW *et al.* (1996) Influence of *Escherichia coli* on motility parameters of human spermatozoa in vitro. *Int J Androl* 19, 271–277.

Diemer T, Hales DB & Weidner W. (2003a) Immune-endocrine interactions and Leydig cell function: the role of cytokines. *Andrologia* 35, 55–63.

Diemer T, Huwe P, Ludwig M *et al.* (2003b) Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters in vitro. *Andrologia* 35, 100–105.

Dieterle S. (2008) Urogenital infections in reproductive medicine. *Andrologia* 40, 117–119.

Dijkstra CD, Döpp EA, Joling P & Kraal G. (1985) The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology* 54, 589–599.

Dimitrov DG & Petrovská M. (1996) Effects of products of activated immune cells and recombinant cytokines on spontaneous and ionophore-induced acrosome reaction. *Am J Reprod Immunol* 36, 150–156.

Dohle GR. (2003) Inflammatory-associated obstructions of the male reproductive tract. *Andrologia* 35, 321–324.

Dondero F, Rossi T, D'Offizi G *et al.* (1996) Semen analysis in HIV seropositive men and in subjects at high risk for HIV infection. *Hum Reprod* 11, 765–768.

Dousset B, Hussenet F, Daudin M *et al.* (1997) Seminal cytokine concentrations (IL-1beta, IL-2, IL-6, sR IL-2, sR IL-6), semen parameters and blood hormonal status in male infertility. *Hum Reprod* 12, 1476–1479.

Durazzo M, Premoli A, Di Bisceglie C *et al.* (2006) Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? *World J Gastroenterol* 12, 3073–3076.

Eggert-Kruse W, Hofmann H, Gerhard I *et al.* (1988) Effects of antimicrobial therapy on sperm-mucus interaction. *Hum Reprod* 3, 861–869.

Eggert-Kruse W, Boit R, Rohr G *et al.* (2001) Relationship of seminal plasma interleukin (IL) -8 and IL-6 with semen quality. *Hum Reprod* 16, 517–528.

Eickhoff R, Baldauf C, Koyro HW *et al.* (2004) Influence of macrophage migration inhibitory factor (MIF) on the zinc content and redox state of protein-bound sulphydryl groups in rat sperm: indications for a new role of MIF in sperm maturation. *Mol Hum Reprod* 10, 605–611.

Eisermann J, Register KB, Strickler RC *et al.* (1989) The effect of tumor necrosis factor on human sperm motility in vitro. *J Androl* 10, 270–274.

Eley A, Pacey AA, Galdiero M *et al.* (2005) Can *Chlamydia trachomatis* directly damage your sperm? *Lancet Infect Dis* 5, 53–57.

Estrada LS, Champion HC, Wang R *et al.* (1997) Effect of tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) on human sperm motility, viability and motion parameters. *Int J Androl* 20, 237–242.

Evenson DP, Larson KL & Jost LK. (2002) Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with the other techniques. *J Androl* 23, 25–43.

Fedder J & Ellerman-Eriksen S. (1995) Effect of cytokines on sperm motility and ionophore-stimulated acrosome reaction. *Arch Androl* 35, 173–185.

Fijak M & Meinhardt A. (2006) The testis in immune privilege. *Immunol Rev* 213, 66–81.

Foresta C, Garolla A, Zuccarello D *et al.* (2010a) Human papillomavirus found in sperm head of young adult males affects the progressive motility. *Fertil Steril* 93, 802–806.

Foresta C, Pizzol D, Moretti A *et al.* (2010b) Clinical and prognostic significance of human papillomavirus DNA in the sperm or exfoliated cells of infertile patients and subject with risk factors. *Fertil Steril* 94, 1723–1727. [Epub ahead of print].

Fraczek M, Sanocka D, Kamieniczna M *et al.* (2008) Proinflammatory cytokines as an intermediate factor enhancing lipid sperm membrane peroxidation in in vitro conditions. *J Androl* 29, 85–92.

Frenette G, Légaré C, Saez F *et al.* (2005) Macrophage migration inhibitory factor in the human epididymis and semen. *Mol Hum Reprod* 11, 575–582.

Gallegos G, Ramos B, Santiso R *et al.* (2008) Sperm DNA fragmentation in infertile men with genitourinary infection by *Chlamydia trachomatis* and Mycoplasma. *Fertil Steril* 90, 328–334.

Gallegos-Avila G, Ortega-Martínez M, Ramos-González B *et al.* (2009) Ultrastructural findings in semen samples of infertile men infected with *Chlamydia trachomatis* and mycoplasmas. *Fertil Steril* 91, 915–919.

Garrido N, Meseguer M, Remohí J *et al.* (2005) Semen characteristics in human immunodeficiency virus (HIV)- and hepatitis C (HCV)-seropositive males: predictors of the success of viral removal after sperm washing. *Hum Reprod* 20, 1028–1034.

Gdoura R, Kchaou W, Chaari C *et al.* (2007) *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. *BMC Infect Dis* 7, 129.

Gdoura R, Kchaou W, Ammar-Keskes L *et al.* (2008) Assessment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, and *Mycoplasma genitalium* in semen and first void urine specimens of asymptomatic male partners of infertile couples. *J Androl* 29, 198–206.

Gerdprasert O, O'Bryan MK, Muir JA, Caldwell AM, Schlatt S, de Kretser DM & Hedger MP. (2002) The response of testicular leukocytes to lipopolysaccharide-induced inflammation: further evidence for heterogeneity of the testicular macrophage population. *Cell Tissue Res* 308, 277–285.

Gopalkrishnan K, Hinduja IN & Kumar TC. (1990) Semen characteristics of asymptomatic males affected by *Trichomonas vaginalis*. *J In Vitro Fert Embryo Transf* 7, 165–167.

Gregoriou O, Botsis D, Papadias K *et al.* (1989) Culture of seminal fluid in infertile men and relationship to semen evaluation. *Int J Gynaecol Obstet* 28, 149–153.

Griveau JF & Le Lannou D. (1997) Reactive oxygen species and human spermatozoa: physiology and pathology. *Int J Androl* 20, 61–69.

Gruschwitz MS, Brezinschek R & Brezinschek HP. (1996) Cytokine levels in the seminal plasma of infertile males. *J Androl* 17, 158–163.

Guermonprez P, Valladeau J, Zitvogel L, Thery C & Amigorena S. (2002) Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 20, 621–667.

Hakimi H, Geary I, Pacey A *et al.* (2006) Spermicidal activity of bacterial lipopolysaccharide is only partly due to lipid A. *J Androl* 27, 774–779.

Han Q, Liu J, Wang T *et al.* (2004) Influence of the metabolite produced by *Trichomonas vaginalis* on human sperm motility in vitro. *Zhonghua Nan Ke Xue* 10, 272–274.

Haney AF, Hughes SF & Weinberg JB. (1992) The lack of effect of tumor necrosis factor-alpha, interleukin-1-alpha, and interferon-gamma on human sperm motility in vitro. *J Androl* 13, 249–253.

Hedger MP. (2002) Macrophages and the immune responsiveness of the testis. *J Reprod Immunol* 57, 19–34.

Hedger MP & Meinhardt A. (2003) Cytokines and the immune-testicular axis. *J Reprod Immunol* 58, 1–26.

Henkel R, Kierspel E, Hajimohammad M *et al.* (2003) DNA fragmentation of spermatozoa and assisted reproduction technology. *Reprod Biomed Online* 7, 477–484.

Hill JA, Haimovici F, Politch JA *et al.* (1987) Effects of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. *Fertil Steril* 47, 460–465.

Hill JA, Cohen J & Anderson DJ. (1989) The effects of lymphokines and monokines on human sperm fertilizing ability in the zona-free hamster egg penetration test. *Am J Obstet Gynecol* 160 (5 Pt 1): 1154–1159.

Hofstetter A, Schmiedt E, Schill WB *et al.* (1978) Genital mycoplasma strains as a cause of male infertility. *Helv Chir Acta* 45, 329–333.

Hooper P, Smythe E, Richards RC, Howard CV, Lynch RV & Lewis-Jones DI. (1995) Total number of immunocompetent cells in the normal rat epididymis and after vasectomy. *J Reprod Fertil* 104, 193–198.

Hosseinzadeh S, Brewis IA, Eley A *et al.* (2001) Co-incubation of human spermatozoa with *Chlamydia trachomatis* serovar E causes premature sperm death. *Hum Reprod* 16, 293–299.

Hosseinzadeh S, Pacey AA & Eley A. (2003) *Chlamydia trachomatis*-induced death of human spermatozoa is caused primarily by lipopolysaccharide. *J Med Microbiol* 52 (Pt 3):193–200.

Hosseinzadeh S, Eley A & Pacey AA. (2004) Semen quality of men with asymptomatic chlamydial infection. *J Androl* 25, 104–109.

Huang JM, Huang TH, Qiu HY *et al.* (2002) Studies on the integration of hepatitis B virus DNA sequence in human sperm chromosomes. *Asian J Androl* 4, 209–212.

Huang JM, Huang TH, Qiu HY *et al.* (2003) Effects of hepatitis B virus infection on human sperm chromosomes. *World J Gastroenterol* 9, 736–740.

Hussenet F, Dousset B, Cordonnier JL *et al.* (1993) Tumour necrosis factor alpha and interleukin 2 in normal and infected human seminal fluid. *Hum Reprod* 8, 409–411.

Huwe P, Diemer T, Ludwig M *et al.* (1998) Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. *Andrologia* 30(Suppl. 1), 55–59.

Itoh M, Terayama H, Naito M, Ogawa Y & Tainosho S. (2005) Tissue microcircumstances for leukocytic infiltration into the testis and epididymis in mice. *J Reprod Immunol* 67, 57–67.

Jarecki-Black JC, Lushbaugh WB, Golosov L *et al.* (1988) *Trichomonas vaginalis*: preliminary characterization of a sperm motility inhibiting factor. *Ann Clin Lab Sci* 18, 484–489.

Kalugdan T, Chan PJ, Seraj IM *et al.* (1996) Polymerase chain reaction enzyme-linked immunosorbent assay detection of mycoplasma consensus gene in sperm with low oocyte penetration capacity. *Fertil Steril* 66, 793–797.

Kocak I, Yenisey C, Dundar M *et al.* (2002) Relationship between seminal plasma interleukin-6 and tumor necrosis factor alpha levels with semen parameters in fertile and infertile men. *Urol Res* 30, 263–267.

Kodama H, Yamaguchi R, Fukuda J *et al.* (1997) Increased deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril* 65, 519–524.

Köhn FM, Erdmann I, Oeda T *et al.* (1998) Influence of urogenital infections on sperm functions. *Andrologia* 30(Suppl. 1), 73–80.

Kokab A, Akhondi MM, Sadeghi MR *et al.* (2010) Raised inflammatory markers in semen from men with asymptomatic chlamydial infection. *J Androl* 31, 114–120.

Kranjcić-Zec I, Dzamić A, Mitrović S *et al.* (2004) The role of parasites and fungi in secondary infertility. *Med Pregl* 57, 30–32.

Krieger JN, Coombs RW, Collier AC *et al.* (1991) Fertility parameters in men infected with human immunodeficiency virus. *J Infect Dis* 164, 464–469.

Lai YM, Yang FP & Pao CC. (1996) Human papillomavirus deoxyribonucleic acid and ribonucleic acid in seminal plasma and sperm cells. *Fertil Steril* 65, 1026–1030.

Lai YM, Lee JF, Huang HY *et al.* (1997) The effect of human papillomavirus infection on sperm cell motility. *Fertil Steril* 67, 1152–1155.

Lampiao F & du Plessis SS. (2008) TNF-alpha and IL-6 affect human sperm function by elevating nitric oxide production. *Reprod Biomed Online* 17, 628–631.

Lampiao F & du Plessis SS. (2009) Effects of tumour necrosis factor alpha and interleukin-6 on progesterone and calcium ionophore-induced acrosome reaction. *Int J Androl* 32, 274–277.

Lanzafame F, La Vignera S, Vicari E *et al.* (2009) Oxidative stress and antioxidant medical treatment in male infertility. *Reprod Biomed Online* 19, 638–659.

Lee CA, Huang CT, King A *et al.* (2002) Differential effects of human papillomavirus DNA types on p53 tumor-suppressor gene apoptosis in sperm. *Gynecol Oncol* 85, 511–516.

van Leeuwen E, Wit FW, Prins JM *et al.* (2008a) Semen quality remains stable during 96 weeks of untreated human immunodeficiency virus-1 infection. *Fertil Steril* 90, 636–641.

van Leeuwen E, Wit FW, Repping S *et al.* (2008b) Effects of antiretroviral therapy on semen quality. *AIDS* 22, 637–642.

Lewis SE, Donnelly ET, Sterling ES *et al.* (1996) Nitric oxide synthase and nitrite production in human spermatozoa: evidence that endogenous nitric oxide is beneficial to sperm motility. *Mol Hum Reprod* 2, 873–878.

Liu JH, Li HY, Cao ZG *et al.* (2002) Influence of several uropathogenic microorganisms on human sperm motility parameters in vitro. *Asian J Androl* 4, 179–182.

Lorusso F, Palmisano M, Chironna M *et al.* (2010) Impact of chronic viral diseases on semen parameters. *Andrologia* 42, 121–126.

Ludwig M, Vidal A, Diemer T, Pabst W, Failing K & Weidner W. (2002) Seminal secretory capacity of the male accessory sex glands in chronic pelvic pain syndrome (CPPS)/chronic prostatitis with special focus on the new prostatitis classification. *Eur Urol* 42, 24–28.

Ludwig M, Vidal A, Diemer T, Pabst W, Failing K & Weidner W. (2003) Chronic prostatitis/chronic pelvic pain pyndrome): seminal markers of inflammation. *World J Urol* 21, 82–85.

Lutz MB & Schuler G. (2002) Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol* 23, 445–449.

Ma J & Xu C. (2004) Relationship between mycoplasma infection and germ cell sulfogalactosylglycerolipid. *Zhonghua Nan Ke Xue* 10, 215–217.

Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zalata A *et al.* (1998) Seminal plasma alpha-glucosidase activity and male infertility. *Hum Reprod* 13, 591–595.

Marconi M, Pilatz A, Wagenlehner F, Diemer T & Weidner W. (2009) Impact of infection on the secretory capacity of the male accessory glands. *Int Braz J Urol* 35, 299–308.

Martínez P, Proverbio F & Camejo MI. (2007) Sperm lipid peroxidation and pro-inflammatory cytokines. *Asian J Androl* 9, 102–107.

Meares EM & Stamey TA. (1968) Bacteriologic localization patterns in bacterial prostatitis and urethritis. *Invest Urol* 5, 492–518.

Meinhardt A & Hedger MP. (2011) Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol Cell Endocrinol* 335, 60–68.

Mellman I & Steinman RM. (2001) Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106, 255–258.

Moretti E, Federico MG, Giannerini V *et al.* (2008) Sperm ultrastructure and meiotic segregation in a group of patients with chronic hepatitis B and C. *Andrologia* 40, 286–291.

Muciaccia B, Corallini S, Vicini E *et al.* (2007) HIV-1 viral DNA is present in ejaculated abnormal spermatozoa of seropositive subjects. *Hum Reprod* 22, 2868–2878.

Naito M & Itoh M. (2008) Patterns of infiltration of lymphocytes into the testis under normal and pathological conditions in mice. *Am J Reprod Immunol* 59, 55–61.

Nickel JC. (1997) The pre and post massage test (PPMT): a simple screen for prostatitis. *Tech Urol* 3, 38–43.

Nicopoullos JDM, Almeida PA, Ramsay JWA *et al.* (2004) The effect of human immunodeficiency virus on sperm parameters and the outcome of intrauterine insemination following sperm washing. *Hum Reprod* 19, 2289–2297.

Núñez-Calonge R, Caballero P, Redondo C *et al.* (1998) *Ureaplasma urealyticum* reduces motility and induces membrane alterations in human spermatozoa. *Hum Reprod* 13, 2756–2761.

O'Bryan MK, Gerdprasert O, Nikolic-Paterson DJ, Meinhardt A, Muir JA, Foulds LM, Phillips DJ, de Kretser DM & Hedger MP. (2005) Cytokine profiles in the testes of rats treated with lipopolysaccharide reveal localized suppression of inflammatory responses. *Am J Physiol Regul Integr Comp Physiol* 288, R1744–R1755.

Ochsendorf FR. (1999) Infections in the male genital tract and reactive oxygen species. *Hum Reprod Update* 5, 399–420.

Okamura N, Tajima Y, Ishikawa H, Yoshii S, Koiso K & Sugita Y. (1986) Lowered levels of bicarbonate in seminal plasma cause the poor sperm motility in human infertile patients. *Fertil Steril* 45, 265–272.

Omu AE, Al-Qattan F, Al-Abdul-Hadi FM *et al.* (1999) Seminal immune response in infertile men with leukocytospermia: effect on antioxidant activity. *Eur J Obstet Gynecol Reprod Biol* 86, 195–202.

Perdichizzi A, Nicoletti F, La Vignera S *et al.* (2007) Effects of tumour necrosis factor-alpha on human sperm motility and apoptosis. *J Clin Immunol* 27, 152–162.

Pèrez-Andino J, Buck CB & Ribbeck K. (2009) Adsorption of human papillomavirus 16 to live human sperm. *PLoS ONE* 4, e5847.

Polfliet MMJ, Fabriek BO, Daniëls WP, Dijkstra CD & van den Berg TK. (2006) The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production. *Immunobiology* 211, 419–425.

Pöllänen P & Cooper TG. (1994) Immunology of the testicular excurrent ducts. *J Reprod Immunol* 26, 167–216.

Prabha V, Sandhu R, Kaur S *et al.* (2010) Mechanism of sperm immobilization by *Escherichia coli*. *Adv Urol* 2010, 240–268. Epub 2010 Mar 30.

Purvis K & Christiansen E. (1993) Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 16, 1–13.

Reichart M, Kahane I & Bartoov B. (2000) In vivo and in vitro impairment of human and ram sperm nuclear chromatin integrity by sexually transmitted *Ureaplasma urealyticum* infection. *Biol Reprod* 63, 1041–1048.

Rennemeier C, Frambach T, Hennicke F *et al.* (2009) Microbial quorum-sensing molecules induce acrosome loss and cell death in human spermatozoa. *Infect Immun* 77, 4990–4997.

Rintala MA, Grènman SE, Pöllänen PP *et al.* (2004) Detection of high-risk HPV DNA in semen and its association with the quality of semen. *Int J STD AIDS* 15, 740–743.

Rival C, Lustig L, Iosub R, Guazzone VA, Schneider E, Meinhardt A & Fijak M. (2006) Identification of a dendritic cell population in normal testis and in chronically inflamed testis of rats with autoimmune orchitis. *Cell Tissue Res* 324, 311–318.

Rival C, Guazzone VA, von Wulffen W, Hackstein H, Schneider E, Lustig L, Meinhardt A & Fijak M. (2007) Expression of co-stimulatory molecules, chemokine receptors and proinflammatory cytokines in dendritic cells from normal and chronically inflamed rat testis. *Mol Hum Reprod* 13, 853–861.

Rodrigues A, Queiróz DB, Honda L, Silva EJ, Hall SH & Avellar MC. (2008) Activation of toll-like receptor 4 (TLR4) by in vivo and in vitro exposure of rat epididymis to lipopolysaccharide from *Escherichia coli*. *Biol Reprod* 79, 1135–1147.

Rose BI & Scott B. (1994) Sperm motility, morphology, hyperactivation, and ionophore-induced acrosome reactions after overnight incubation with mycoplasmas. *Fertil Steril* 61, 341–348.

Rosemond A, Lanotte P, Watt S *et al.* (2006) Systematic screening tests for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in urogenital specimens of infertile couples. *Pathol Biol (Paris)* 54, 125–129.

Rowe P, Comhaire F, Hargreave TB & Mellows HJ. (1993) (eds) World Health Organization Manual for the Standardised Investigation and Diagnosis of the Infertile Couple. Cambridge University Press, Cambridge.

Said TM, Agarwal A, Falcone T *et al.* (2005) Infliximab may reverse the toxic effects induced by tumor necrosis factor alpha in human spermatozoa: an in vitro model. *Fertil Steril* 83, 1665–1673.

Sainio-Pöllänen S, Saari T, Simell O & Pöllänen P. (1996) CD28-CD80/CD86 interactions in testicular immunoregulation. *J Reprod Immunol* 31, 145–163.

Sanocka D, Jedrzejczak P, Szumała-Kaekol A *et al.* (2003) Male genital tract inflammation: The role of selected interleukins in regulation of pro-oxidant and antioxidant enzymatic substances in seminal plasma. *J Androl* 24, 448–455.

Sanocka-Maciejewska D, Ciupińska M & Kurpisz M. (2005) Bacterial infection and semen quality. *J Reprod Immunol* 67, 51–56.

Saraniya A, Koner BC, Doureradjou P *et al.* (2008) Altered malondialdehyde, protein carbonyl and sialic acid levels in seminal plasma of microscopically abnormal semen. *Andrologia* 40, 56–57.

Satta A, Stivala A, Garozzo A *et al.* (2006) Experimental *Chlamydia trachomatis* infection causes apoptosis in human sperm. *Hum Reprod* 21, 134–137.

Schulz M, Sànchez R, Soto L *et al.* (2010) Effect of *Escherichia coli* and its soluble factors on mitochondrial membrane potential, phosphatidylserine traslocation, viability and motility of human spermatozoa. *Fertil Steril* 294, 619–623.

Sergerie M, Martinet S, Kiffer N *et al.* (2004) Impact of reverse transcriptase inhibitors on sperm mitochondrial genomic DNA in assisted reproduction techniques. *Gynecol Obstet Fertil* 32, 841–849.

Shalhoub D, Abdel-Latif A, Fredericks CM *et al.* (1986) Physiological integrity of human sperm in the presence of *Ureaplasma urealyticum*. *Arch Androl* 16, 75–80.

Shang XJ, Huang YF, Xiong CL *et al.* (1999) *Ureaplasma urealyticum* infection and apoptosis of spermatogenic cells. *Asian J Androl* 1, 127–129.

Sigal LJ & Rock KL. (2000) Bone marrow-derived antigen-presenting cells are required for the generation of cytotoxic T lymphocyte responses to viruses and use transporter associated with antigen presentation (TAP)-dependent and -independent pathways of antigen presentation. *J Exp Med* 192, 1143–1150.

Sikka SC, Champion HC, Bivalacqua TJ *et al.* (2001) Role of genitourinary inflammation in infertility: synergistic effects of lipopolysaccharide and interferon-gamma on human spermatozoa. *Int J Androl* 24, 136–141.

Sikorski R, Kapec E, Krzeminski A *et al.* (2001) Levels of proinflammatory cytokines (Il-1 alpha, Il-6, TNF-alpha) in the semen plasma of male partners of infertile couples. *Ginekol Pol* 72, 1325–1328.

Soffer Y, Ron-El R, Golan A *et al.* (1990) Male genital mycoplasmas and *Chlamydia trachomatis* culture: its relationship with accessory gland function, sperm quality, and autoimmunity. *Fertil Steril* 53, 331–336.

Steinman RM. (1991) The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 9, 271–296.

Stubbs AC, Martin KS, Coeshott C, Skaates SV, Kuritzkes DR, Bellgrau D, Franzusoff A, Duke RC & Wilson CC. (2001) Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nat Med* 7, 625–629.

Suescun MO, Rival C, Theas MS, Calandra RS & Lustig L. (2003) Involvement of tumor necrosis factor-alpha in the pathogenesis of autoimmune orchitis in rats. *Biol Reprod* 68, 2114–2121.

Suleiman SA, Ali ME, Zaki ZM *et al.* (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E. *J Androl* 17, 530–537.

Svenstrup HF, Fedder J, Abraham-Peskir J *et al.* (2003) *Mycoplasma genitalium* attaches to human spermatozoa. *Hum Reprod* 18, 2103–2109.

Talkington DF, Davis JK, Canupp KC *et al.* (1991) The effects of three serotypes of *Ureaplasma urealyticum* on spermatozoal motility and penetration in vitro. *Fertil Steril* 55, 170–176.

Tanaka H, Karube A, Kodama H *et al.* (2000) Mass screening for human papillomavirus type 16 infection in infertile couples. *J Reprod Med* 45, 907–911.

Tian Y-H, Xiong JW, Hu L *et al.* (2007) *Candida albicans* and filtrates interfere with human spermatozoal motility and alter the ultrastructure of spermatozoa: an in vitro study. *Int J Androl* 30, 421–429.

Tuttle JP Jr, Holbrook TW & Derrick FC. (1977) Interference of human spermatozoal motility by *Trichomonas vaginalis*. *J Urol* 118, 1024–1025.

Tuttle JP Jr, Bannister ER & Derrick FC. (1997) Interference of human spermatozoal motility and spermatozoal agglutination by *Candida albicans*. *J Urol* 118, 797–799.

Twigg JP, Irvine DS & Aitken RJ. (1998) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum Reprod* 13, 1864–1871.

Umapathy E. (2005) STD/HIV association: effects on semen characteristics. *Arch Androl* 51, 361–365.

Upadhyaya M, Hibbard BM & Walker SM. (1984) The effect of *Ureaplasma urealyticum* on semen characteristics. *Fertil Steril* 41, 304–308.

Vicari E. (1999) Seminal leukocyte concentration and related specific reactive oxygen species production in patients with male accessory gland infections. *Hum Reprod* 14, 2025–2030.

Vicari E. (2000) Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. *Hum Reprod* 15, 2536–2544.

Vicari E & Calogero AE. (2001) Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. *Hum Reprod* 16, 2338–2342.

Vicari E, La Vignera S & Calogero AE. (2002) Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertil Steril* 78, 1203–1208.

Vicari E, Arcoria D, Di Mauro C *et al.* (2006a) Sperm output in patients with primary infertility and hepatitis B or C virus; negative influence of HBV infection during concomitant varicocele. *Minerva Med* 97, 65–77.

Vicari E, La Vignera S, Castiglione R *et al.* (2006b) Sperm parameters abnormalities, low seminal fructose and reactive oxygen species overproduction do not discriminate patients with unilateral or bilateral post-infectious inflammatory prostato-vesiculo-epididymitis. *J Endocrinol Invest* 29, 18–25.

Villegas J, Schulz M, Soto L *et al.* (2005) Bacteria induce expression of apoptosis in human spermatozoa. *Apoptosis* 10, 105–110.

Wan CC, Wang H, Hao BJ *et al.* (2003) Infection of *Chlamydia trachomatis* and apoptosis of spermatogenic cells. *Zhonghua Nan Ke Xue* 9, 350–351.

Wang YF & Holstein AF. (1983) Intraepithelial lymphocytes and macrophages in the human epididymis. *Cell Tissue Res* 233, 517–521.

Wang Y, Kang L, Hou Y *et al.* (2005) Microelements in seminal plasma of infertile men infected with *Ureaplasma urealyticum*. *Biol Trace Elem Res* 105, 11–18.

Wang Y, Liang CL, Wu JQ *et al.* (2006) Do *Ureaplasma urealyticum* infections in the genital tract affect semen quality? *Asian J Androl* 8, 562–568.

Weidner W, Krause W & Ludwig M. (1999) Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Hum Reprod Update* 5, 421–432.

Weidner W, Colpi GM, Hargreave TB *et al.* (2002) EAU Guidelines on male infertility. *Eur Urol* 42, 313–322.

Weidner W, Wagenlehner FM, Marconi M, Pilatz A, Pantke KH & Diemer T. (2008) Acute bacterial prostatitis and chronic prostatitis/chronic pelvic pain syndrome: andrological implications. *Andrologia* 40, 105–112.

Wincek TJ, Meyer TK, Meyer MR *et al.* (1991) Absence of a direct effect of recombinant tumor necrosis factor-alpha on human sperm function and murine preimplantation development. *Fertil Steril* 56, 332–339.

Witkin SS, Jeremias J, Bongiovanni AM & Munoz MG. (1996) Immune regulation in the male genital tract. *Infect Dis Obstet Gynecol* 4, 131–135.

Wolff H, Bezold G, Zebhauser M & Meurer M. (1991) Impact of clinically silent inflammation on male genital tract organs as reflected by biochemical markers in semen. *J Androl* 12, 331–334.

World Health Organization (2000) WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male, 2nd edn. Cambridge University Press, New York.

Xu C, Lu MG, Feng JS *et al.* (2001) Germ cell apoptosis induced by *Ureaplasma urealyticum* infection. *Asian J Androl* 3, 199–204.

Yeung CH, Nashan D, Sorg C, Oberpenning F, Schulze H, Nieschlag E & Cooper TG. (1994) Basal cells of the human epididymis-antigenic and ultrastructural similarities to tissue-fixed macrophages. *Biol Reprod* 50, 917–926.

Zheng J, Yu SY, Jia DS *et al.* (2008) *Ureaplasma urealyticum* infection in the genital tract reduces seminal quality in infertile men. *Zhonghua Nan Ke Xue* 14, 507–512.

Zhou XL, Sun PN, Huang TH *et al.* (2009) Effects of hepatitis B virus S protein on human sperm function. *Hum Reprod* 24, 1575–1583.