Review Article

# Impact of Inflammation on Male Reproductive Tract

Alfred Azenabor, Ayodele Oloruntoba Ekun, Oluyemi Akinloye *

- *Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria*

* *Corresponding Author:* Oluyemi Akinloye, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria
*E-mail:* oakinloye@unilag.ng.edu, oluyemiakinloye@hotmail.com

**Received:** Oct. 7, 2014
**Accepted:** Jan. 21, 2015

## Abstract

Fertility in the male is dependent on the proper production of sperm cells. This process, called spermatogenesis is very complex and involves the synchronization of numerous factors. The presence of pro–inflammatory cytokines, tumor necrosis factor-alpha (TNF–α), interleukin–1 alpha (IL–1 α) and interleukin 1 beta (IL–1 β) cytokines in the male reproductive tract (testis, epididymis and sperm) may have certain physiological functions. However, when the levels of these cytokines are higher than normal, as seen in conditions of inflammation, they become very harmful to sperm production. Moreover, inflammation is also associated with oxidative stress and the latter is well known to impair sperm function. Epidemiological studies regarding male infertility have revealed that more and more infertile men suffer from acute or chronic inflammation of the genitourinary tract, which often occurs without any symptoms. The inflammatory reactions within the male genital tract are inevitably connected with oxidative stress. Oxidative stress, especially in sperm, is harmful because it damages sperm DNA and causes apoptosis in sperm. This article reviewed the suggested mechanisms and contribution of inflammation to male infertility. In addition, the review was further strengthened by discussing how inflammation affects both fertility and assisted reproductive technologies (ART).

**Keywords:** Cytokines, Fertility, Infertility, Inflammation, Spermatogenesis.
**To cite this article:** Azenabor A, Ekun AO, Akinloye O. Impact of Inflammation on Male Reproductive Tract. J Reprod Infertil. 2015;16(3):123-129.

## Introduction

Spermatogenesis is the process by which a complex, interdependent population of germ cells produces spermatozoa by mitosis and meiosis. The process of spermatogenesis takes place within the coiled seminiferous tubules, and continues from puberty to old age. It consists of various stages beginning with the formation of germ cells (spermatogonia) in the germinal epithelium followed by their progressive development into primary and secondary spermatocytes, and finally mature spermatozoa. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. DNA methylation and histone modification have been implicated in the regulation of this process (1). The process of spermatogenesis is highly organized; proliferation and differentiation of the male germ cells and the intratesticular and extratesticular mechanisms of regulation of spermatogenesis can be disturbed at every level (2). This may occur as a result of environmental influences or may be due to diseases that directly or indirectly affect spermatogenesis, leading to infertility in men. Male factors have been reported to contribute between 30-55% (3-6) to global cases of infertility. Poongothai et al. (2009) reported that male factor contributes averagely to half of the cases of infertility in couples (7). Male factor infertility is seen as an alteration in sperm concentration and/or motility or morphology in at least one sample of two sperm analyses, collected between 1 and 4 weeks (8). Apart from known hormonal disturbances (9), other physical and psychological problems may also contribute to male infertility. These include environmental factors such as diet and toxic elements

(10-11), genetic aberrations (12-13), infection and inflammation (14) *etc*. Infection remains the commonest cause of infertility in Africa particularly in sub-Saharan Africa (15-16). When there is an infection to the reproductive tract, one important function of the innate immune system is to recruit more phagocytic cells and effector molecules to the site of the infection through the release of a battery of cytokines and other inflammatory mediators that have profound effects on subsequent events.

***Causes of inflammation in the male reproductive tract:*** There are several causes of inflammation in the male reproductive tract. These include (i) ejaculatory duct obstruction: This is a common cause of male infertility and infections have been reported to be present in at least 20-50% of these men (17). (ii) Epididymitis: This is the inflammation of the epididymis, a structure in the male reproductive system that joins the testes and the vas deferens. Inflammation of the epididymis may result in further complications such as scrotal swelling, pain, penile discharge and blood in urine. (iii) Infections: Inflammation results from sexually transmitted infections *e.g.* gonorrhoea, Chlamydia, *Escherichia coli* was reported to be the main cause of epididymitis in older men, but other types of bacteria including mycobacteria and ureaplasma may also cause this condition. (iv) Urethritis: There could also be bladder or urethral infection that moves to the epididymis. In children, mumps and other viral infections may cause epididymitis. (v). Testicular torsion: This is a common problem affecting fertility that is caused by a supportive tissue abnormality which allows the testes to twist inside the scrotum which is characterized by extreme swelling. Torsion pinches the blood vessels that feed the testes shut which causes testicular damage. (vi) Varicocoele: This is an enlargement of the internal spermatic veins that drain blood from the testicle to the abdomen (back to the heart). A varicocoele develops when the one way valves in the spermatic veins are damaged causing an abnormal back flow of blood from the abdomen into the scrotum creating a hostile environment for sperm development. (vii) Several other causes include male urogenital obstruction, chronic prostatitis, inflammation of one or both testes (orchitis), and drug therapy.

It is important to note that when any or the above processes occur in the male genital tract, the immune system will respond to the pathogens and possible tissue damage. The collateral damage caused by this type of inflammation usually accumulates slowly, sometimes asymptomatically for years and further leads to tissue deterioration (18). The inflammatory system can be linked to a sprinkler system that prevents fire from spreading in a building. While the intention of the process is to limit damage and restore function (positive), the response itself can cause significant harm. One undesirable consequence of inflammation, for example, is that some enzymes and toxic products contained within phagocytic tissues are inevitably released, damaging cells and tissues.

***Effects of inflammation on the male reproductive tract:*** Inflammation is the process of responding to injury and tissue damage. This process brings leucocytes and plasma molecules to sites of infection or tissues. Three principal changes occur when there is a case of acute inflammation; an increased blood supply to the affected area, an increase in capillary permeability allowing larger serum molecules to enter the tissues and an increase in leukocyte migration into the tissue. When there is a failure to eliminate the infectious agent, it leads to chronic inflammation. This is characterized with recruitment and activation of macrophages, lymphocytes and other cells which trigger a coordinated action of cytokines. In contrast to acute inflammation, where the host response leads to elimination of the irritant, followed by recovery involving tissue regeneration or repair, chronic inflammation is characterized by inflammation and repair occurring concurrently, rather than consecutively. Note that repair is always a feature of chronic inflammation because it is associated with irritants that cause destruction of tissue architecture. Repair is typically achieved by ingrowth of granulation tissue, which includes macrophages, fibroblasts and new blood vessels (19).

The direct association between acute or chronic inflammation and the development of infertility constitute important issues in contemporary medicine. The reduced semen quality during the inflammatory process can result from impairment of accessory gland functions, obstruction of sperm transport, and dysregulation of spermatogenesis (20-21). Pro–inflammatory cytokines usually act locally, since they are produced by locally activated cells or produced temporarily after the stimulus has been activated. In male gonads, cytokines are also produced physiologically and have been reported to be involved in the normal function of the organ (22-23). In this respect, they appear as the natural components of seminal plasma (24).

The main source of cytokines in the male gonad is testicular macrophages, although some cytokines (IL–1 and IL-6) were also observed by some authors to be produced by Leydig and Sertoli cells (25). Cytokines, particularly TNF–α, controls movement of leukocytes into tissues when there is tissue damage. TNF–α is produced primarily by macrophages and other mononuclear phagocytes and has many functions in the development of inflammation and the activation of other leukocytes. Notably, TNF–α induces adhesion molecules and chemokines on the endothelium and activates the microbial systems of phagocytes. In addition, TNF–α induces apoptosis. IL–1 is another important inflammatory cytokine and shares functions with TNF–α (26).

Inflammatory responses, which are operationally characterized by pain, redness, heat and swelling at the site of infection in the male genital tract reflect two types of changes in the seminal vesicles. The first of these is an increase in diameter, leading to an increased local seminal flow–hence the heat and redness and a reduction in the velocity of seminal flow, especially along the surface of local seminal vesicles.

Inflammatory damage on the male genital tract leads to the increased generation of reactive oxygen species (ROS). In addition, ROS or free radical overproduction associated with inflammatory reactions may be caused by pathological, bacterial strains that colonize the reproductive tract (27-28). Free radicals are group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify bio-mole cules they encounter. Reacting almost immediately with any substance in their environment, results in chains of reaction leading to cellular damage (29). A study by Agarwal et al. (2003) showed superoxide, hydroxyl and hydrogen hydroxide radicals as the major ROS present in seminal plasma (30).

To maintain normal cell function, excess ROS must be continuously inactivated by seminal plasma antioxidants. Oxidative stress arises when excess free radicals overwhelm the antioxidant defence of the male reproductive tract (31-32), thereby damaging male reproductive tract. It is interesting to note that seminal oxidative stress was earlier reported to correlate negatively with sperm concentration, motility and function (33). These observations also suggest that cytokines, mediators of oxidative damage may also have an impact on the quality of semen and male fertility.

The participation of some cytokines in the regulation of fertility is dependent upon their concentration; for example, a study by Naz and Evans (1998) showed that interleukin 12 correlated with sperm density and sperm morphology (34). Increased levels of interleukin 6 were also observed in infertile men by the same author in an earlier study conducted. High levels of certain cytokines in semen are often linked with a decrease in the quality of the seminological parameters (35).

More recent studies have shown increased TNF–α in semen to be associated with reduced sperm count, sperm motility, and sperm morphology (36-37). The increased levels of this cytokine cause apoptosis in semen as a result of proliferation and differentiation of beta cells, proliferation of T cells and natural killer cells. Interleukin1 alpha (IL–1 α) and interleukin1 beta (IL1–β) also induce apoptosis in semen via proliferation and differentiation of beta cells, chemoattraction of leucocytes to the site of inflammation and induction of neutrophils and monocyte generation. These cytokines were also observed to adversely affect quality of semen. In another study reported by the same author, increased levels of IL–1 β were associated with a decrease in sperm motility (38). These results were associated with a simultaneous increase in seminal levels of reactive oxygen species as well as malondiadehyde (MDA); a product of lipid peroxidation, consequent to oxidative damage. The same cytokines that act as elements of immune modulation for the male gonad appear in large concentrations in semen during infection and when the tissues are damaged. Their participation in inflammation is closely connected with the accompanying leukocytospermia (39-40). The clinical significance of increased numbers of semen leukocytes remains a subject of controversy. While some studies have associated with disturbed spermatogenesis (41-42), others have linked it to harmful influence of environmental factors (43), and atypical sexual behavior (44). Under inflammatory conditions, white blood cells (WBC) in the semen leak from the site of infection or inflammation. In normal conditions, leukocytes are restricted to the center of seminal vesicles, where the flow is fastest. In inflammatory sites, the blood vessels feeding the testis are dilated allowing the leukocytes to move out of the center of the blood vessels and interact with the vascular endothelium. In addition to these changes, there is an increase in permeability, leading to the local accumulation of fluid–hence the swelling and pain

as well as the accumulation of immunoglobulins, complement and other semen proteins in the genital tract. The migration of leukocytes out of the seminal vesicles depends on adhesive interactions activated by the release of inflammatory mediators. Thus, infection or physical damage to male genital tract sets in motion the recruitment of phagocytic cells to the site of damage. Neutrophils exposed to TNF–α are activated to mediate a respiratory burst that generates oxygen radicals and nitric oxide, and release their stored granule contents, contributing both to host defence and to local tissue destruction. In a study designed to evaluate the clinical relevance of inflammatory cytokines, such as TNF–α and IL–1β in seminal plasma of infertile men, the levels of these cytokines correlated significantly with leukocyte counts (45).

***Possible therapeutic interventions:*** Levels of ROS can be reduced by augmenting the scavenging capacity of the seminal plasma with antioxidants (46). Administration of antioxidants to infertile men has been reported to reduce oxidative stress in their sperm cells, improving motility, particularly in asthenospermic patients (47). Although the advantage of micronutrients and antioxidant supplementations as interventions in male infertility remains controversial, in a previous study, an inverse relationship of sperm count and viability was seen in serum and seminal zinc levels, respectively in infertile African men (48). Oxidants can also damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (49). Damage to DNA can cause mutations and possibly cancer, and can be reversed by DNA repair mechanism (50-51) while damage to proteins causes enzyme inhibition, denaturation, and protein degradation (52). In sperm cells, lipid peroxidation reduces membrane fluidity, resulting in the inhibition of normal cellular components for fertilization. Patients with high oxidative stress were found to have sperm with multiple genes and double genes DNA strand breaks in their seminal fluid (53). Sperm DNA is usually protected by its compact organization and by antioxidants in the seminal plasma. Spermatozoa themselves are incapable of DNA repair and must depend on the oocyte for repair after fertilization; however, knowledge concerning the process remains limited (54). ROS exposure may result in DNA base modification, production of free base sites, deletions, frame shifts, DNA cross–links and chromosomal aberrations (55-56). Sikka (2004) reported that ROS promoted apoptosis (57), which may lead to a decrease in sperm viability and density. It is not fully established if sperm apoptosis caused by oxidative stress can also be reversed. However, with proper molecular framework of apoptosis, specific apoptosis inhibitors may have a role in promoting germ cell survival. Sphingosine–1-phosphate is an example of such an apoptosis inhibitor. Current research is underway to identify the agents that may manipulate the apoptotic machinery for therapeutic benefits. One should bear in mind that in the context of male infertility, seminal oxidative stress, sperm DNA damage and apoptosis are interlinked and constitute a unified pathogenic mechanism. The impact of these factors, their clinical significance and management options have always been a subject of controversy. Despite few clinical trials reporting the positive effects of antioxidant administration on sperm DNA integrity, such as N-acetyl–cysteine, studies on apoptotic inhibitors have only been evaluated *in vitro*; their efficacy still remains to be validated *in vivo*. Some antioxidants and micronutrients supplementation minimizes endogenous oxidative damage (58). Selenium was reported to protect against sperm DNA damage and is required for normal testicular development, spermatogenesis, motility, and sperm function (59). Selenium, an integral part of the antioxidant enzyme; glutathione peroxidase was implicated to play important role in male fertility in a previous study (60).

***Implication in assisted reproductive technologies:*** Assisted reproductive Technologies (ARTs), which include *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI), currently been used to treat infertility may not be successful when semen samples that contain a high DNA damage are involved. Levels of the oxidative DNA damage marker, 8–hydroxyl–2– deoxyguanosine have been reported to be elevated in infertile men (61). Studies have associated a high rate of miscarriage after ICSI, which possibly reflects the fact that compromised spermatozoa are sometimes used and they lead to irreparable DNA damage in the embryo (62). The degree of inflammations in other genital tract may also affect success rate. In patients with obstructive azoospermia, spermatozoa are often surgically retrieved from epididymis or testicular tissue. In general, surgically extracted spermatozoa tend to have higher

percentages of DNA strand breaks. However, the use of testicular spermatozoa is preferred over epididymal spermatozoa, because the former have less DNA damage and a better developmental potential (63).

## Conclusion

Varieties of disorders ranging from hormonal disturbances to physical problems, to psychological anomaly have been implicated in male infertility. Although many treatment options are now available, in many cases success rate is still low. Often, male infertility is caused by infections and testicular damage with resultant inflammation. Inflammatory reactions within the male genital tract have a negative impact on sperm quality and consequently, infertility. Furthermore, the process of inflammation generates ROS in addition to inherent oxidative stress generated by sperm cells increasing exerted toxic effects on human spermatozoa. The imbalance between prooxidative and antioxidative substances in semen leads to metabolic and functional disorders of the male germ cells and may be a primary cause of some types of infertility. ART may be more successful when markers of oxidative stress and DNA damage in semen, such as 8–hydroxyl–2–deoxyguanosine are evaluated prior to insemination. All possible interventions to abrogate or reduce the consequential effect of inflammation on male reproduction, particularly seminal oxidative stress, sperm DNA damage and apoptosis will not only reduce male reproductive failure but may also increase success rate of assisted reproductive technology.

## Conflict of Interest

Authors declare no conflict of interest.

## References


1. Song N, Liu J, An S, Nishino T, Hishikawa Y, Koji T. Immunohistochemical Analysis of Histone H3 Modifications in Germ Cells during Mouse Spermatogenesis. Acta Histochem Cytochem. 2011;44 (4):183-90.
2. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. Fertil Steril. 2002;77(5):873-82.
3. Belsey MA. The epidemiology of infertility: a review with particular reference to sub-Saharan Africa. Bull World Health Organ. 1976;54(3):319-41.
4. Chukudebelu WO, Esege N, Megafu U. Etiological factors in infertility in Enugu, Nigeria. Infertility. 1979;2(2):193-200.
5. Nwofor AME, Ugezu AI. The difficulties encountered in the management of male infertility in Nnewi, Nigeria. Niger Med J. 2003;45(1):56-9.
6. Ikechebelu JI, Adinma JI, Orie EF, Ikegwuonu SO. High prevalence of male infertility in southeastern Nigeria. J Obstet Gynaecol. 2003;23(6):657-9.
7. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. Singapore Med J. 2009;50(4):336-47.
8. World Health Organisation. WHO Laboratory manual for the examination of human semen-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999. p. 1-86.
9. Akinloye O, Arowojolu AO, Shittu OB, Anetor JI. Cadmium toxicity: a possible cause of male infertility in Nigeria. Reprod Biol. 2006;6(1):17-30.
10. Akinloye O, Akintunde CO, Banjoko SO, Adaramoye AO, Adeleye AO. An assessment of the oestrogenic effect of soyprotein on female rabbits. Food Chem. 2002;77(1):67-9.
11. Akinloye O, Arowojolu AO, Shittu OB, Adejuwon CA, Osotimehin B. Selenium status of idiopathic infertile Nigerian males. Biol Trace Elem Res. 2005;104(1):9-18.
12. Akinloye O, Gromoll J, Callies C, Nieschlag E, Simoni M. Mutation analysis of the X-chromosome linked, testis-specific TAF7L gene in spermatogenic failure. Andrologia. 2007;39(5):190-5.
13. Akinloye O, Gromoll J, Nieschlag E, Simoni M. Androgen receptor gene CAG and GGN polymorphisms in infertile Nigerian men. J Endocrinol Invest. 2009;32(10):797-804.
14. Choudhury SR, Knapp LA. Human reproductive failure I: immunological factors. Hum Reprod Update. 2001;7(2):113-34.
15. Oladokun A, Arulogun O, Oladokun R, Morhason-Bello IO, Bamgboye EA, Adewole IF, et al. Acceptability of child adoption as management option for infertility in Nigeria: evidence from focus group discussions. Afr J Reprod Health. 2009;13 (1):79-91.
16. Dhont N, van de Wijgert J, Luchters S, Muvunyi C, Vyankandondera J, Temmerman M. Sexual violence, HSV-2 and HIV are important predictors for infertility in Rwanda. Hum Reprod. 2010;25(10): 2507-15.
17. Dohle GR, Smit M, Weber RF. Androgens and male fertility. World J Urol. 2003;21(5):341-5.
18. Mitchell RN, Cotran RS, editors. Acute and chronic inflammation. Philadelphia: Saunders Press; 2003. 33 p. (Kumar V, Cotran RS, Robbins SL, editors. Robbins Basic Pathology; vol. 127).

19. DePalma AF, Rothman RH, Lewinnek GE, Canale ST. Anterior interbody fusion for severe cervical disc degeneration. Surg Gynecol Obstet. 1972;134 (5):755-8.
20. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int J Androl. 1993;16 (1):1-13.
21. Comhaire FH, Mahmoud AM, Depuydt CE, Zalata AA, Christophe AB. Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist's viewpoint. Hum Reprod Update. 1999;5(5):393-8.
22. Hales DB, Diemer T, Hales KH. Role of cytokines in testicular function. Endocrine. 1999;10(3):201-17.
23. Soder O, Sultana T, Jonsson C, Wahlgren A, Petersen C, Holst M. The interleukin-1 system in the testis. Andrologia. 2000;32(1):52-5.
24. Diemer T, Hales DB, Weidner W. Immune endocrine interactions and Leydig cell function: the role of cytokines. Andrologia. 2003;35(1):55-63.
25. Maegawa M, Kamada M, Irahara M, Yamamoto S, Yoshikawa S, Kasai Y, et al. A repertoire of cytokines in human seminal plasma. J Reprod Immunol. 2002;54(1-2):33-42.
26. Cudicini C, Lejeune H, Gomez E, Bosmans E, Ballet F, Saez J, et al. Human Leydig cells and Sertoli cells are producers of interleukins-1 and -6. J Clin Endocrinol Metab. 1997;82(5):1426-33.
27. Male D, Brostoff J, Roth DB, Roit I. Mechanism of innate immunity in Immunology. 7th ed. Canada: Elsevier; 2006. 127 p.
28. Keck C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. Hum Reprod Update. 1998;4(6):891-903.
29. Potts JM, Sharma R, Pasqualotto F, Nelson D, Hall G, Agarwal A. Association of ureaplasma urealyticum with abnormal reactive oxygen species levels and absence of leukocytospermia. J Urol. 2000;163 (6):1775-8.
30. Warren JS, Johnson KJ, Ward PA. Oxygen radicals in cell injury and cell death. Pathol Immunopathol Res. 1987;6(5-6):301-15.
31. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79(4):829-43.
32. Sharma RK, Said T, Agarwal A. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian J Androl. 2004;6(2):139-48.
33. Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertil Steril. 2000;74(6):1200-7.
34. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001;8(7):851-62.
35. Naz RK, Evans L. Presence and modulation of interleukin-12 in seminal plasma of fertile and infertile men. J Androl. 1998;19(3):302-7.
36. Gruschwitz MS, Brezinschek R, Brezinschek HP. Cytokine levels in the seminal plasma of infertile males. J Androl. 1996;17(2):158-63.
37. Sanocka D, Jedrzejczak P, Szumała-Kaekol A, Fraczek M, Kurpisz M. Male genital tract inflammation: the role of selected interleukins in regulation of pro-oxidant and antioxidant enzymatic substances in seminal plasma. J Androl. 2003;24(3): 448-55.
38. Kocak I, Yenisey C, Dundar M, Okyay P, Serter M. Relationship between seminal plasma interleukin-6 and tumor necrosis factor alpha levels with semen parameters in fertile and infertile men. Urol Res. 2002;30(4):263-7.
39. Gruschwitz MS, Brezinschek R, Brezinschek HP. Cytokine levels in the seminal plasma of infertile males. J Androl. 1996;17(2):158-63.
40. Shimoya K, Matsuzaki N, Tsutsui T, Taniguchi T, Saji F, Tanizawa O. Detection of interleukin-8 (IL-8) in seminal plasma and elevated IL-8 in seminal plasma of infertile patients with leukospermia. Fertil Steril. 1993;59(4):885-8.
41. Depuydt CE, Bosmans E, Zalata A, Schoonjans F, Comhaire FH. The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. J Androl. 1996;17(6):699-707.
42. Wolff H, Politch JA, Martinez A, Haimovici F, Hill JA, Anderson DJ. Leukocytospermia is associated with poor semen quality. Fertil Steril. 1990;53 (3):528-36.
43. Thomas J, Fishel SB, Hall JA, Green S, Newton TA, Thornton SJ. Increased polymorphonuclear granulocytes in seminal plasma in relation to sperm morphology. Hum Reprod. 1997;12(11):2418-21.
44. Close CE, Roberts PL, Berger RE. Cigarettes, alcohol and marijuana are related to pyospermia in infertile men. J Urol. 1990;144(4):900-3.
45. Blackwell JM, Zaneveld LJ. Effect of abstinence on sperm acrosin, hypoosmotic swelling, and other semen variables. Fertil Steril. 1992;58(4):798-802.
46. Eggert-Kruse W, Kiefer I, Beck C, Demirakca T, Strowitzki T. Role for tumor necrosis factor alpha

(TNF-alpha) and interleukin 1-beta (IL-1beta) determination in seminal plasma during infertility investigation. Fertil Steril. 2007;87(4):810-23.

47. Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. Reprod Biomed Online. 2004;8(6):616-27.
48. Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Hum Reprod. 2011;26 (7):1628-40.
49. Akinloye O, Abbiyesuku FM, Oguntibeju OO, Arowojolu AO, Truter EJ. The impact of blood and seminal plasma zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. Reprod Biol. 2011;11(2):83-98.
50. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997;82(2):291-5.
51. Nakabeppu Y, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T, Nakatsu Y. Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol Chem. 2006;387(4):373-9.
52. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.
53. Stadtman ER. Protein oxidation and aging. Science. 1992;257(5074):1220-4.
54. Twigg J, Fulton N, Gomez E, Irvine DS, Aitken RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Hum Reprod. 1998;13(6): 1429-36.
55. Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reprod Biomed Online. 2003;7(1):65-70.
56. Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertil Steril. 2000;74(6):1200-7.
57. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update. 2003;9(4):331-45.
58. Sikka SC. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. J Androl. 2004;25(1):5-18.
59. Agarwal A, Sekhon LH. The role of antioxidant therapy in the treatment of male infertility. Hum Fertil (Camb). 2010;13(4):217-25.
60. Ursini F, Heim S, Kiess M, Maurine M, Roveri A, Wissing J, et al. Dual function of the selenoprotein PHGPx during sperm maturation. Science. 1999; 285(5432):1393-6.
61. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril. 1997;68(3):519-24.
62. Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol. 2003;101(6):1229-35.
63. Steele EK, McClure N, Maxwell RJ, Lewis SE. A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia. Mol Hum Reprod. 1999;5(9):831-5.