Andrology- Open Access

Micic et al., Andrology 2018, 7:2
DOI: 10.4172/2167-0250.1000197

Research Article Open Access

Sperm Quality and Seminal Biochemical Parameters in Infertile Men with and without Leukocytospermia

Djordjevic D[1], Lalic N[2], Vukovic I[1], Nale D[1], Perovic D[3], Kisic DT[4] and Micic S[2*]

[1]*Clinic of Urology, Clinical Center of Serbia, Belgrade, Serbia*

[2]*Department of Andrology, Uromedica Polyclinic, Belgrade, Serbia*

[3] *Faculty of Pharmacy, Department of Medical Biochemistry, University of Belgrade, Serbia*

[4]*Faculty of Medicine, Institute of Epidemiology, Belgrade, Serbia*

[*]**Corresponding author:** Sava Micic, MD PhD, Department of Andrology, Uromedica Polyclinic, Belgrade, Serbia, Tel: +38163656119, E-mail: uromedica@sbb.rs

Received date: October 2, 2018; **Accepted date:** October 11, 2018; **Published date:** October 18, 2018

Abstract

Objective: White Blood Cells (WBC) are commonly present in almost every human semen sample, but the clinical significance of leukocytospermia, defined as more than 1 × 10^6/mL of WBC in seminal plasma, is not elucidated. The aim of this study was to evaluate the association of the leukocytospermia with sperm characteristics and biochemical markers of function of the male accessory glands in infertile men.

Methods: One hundred and eighty-five men with fertility problems were investigated. They were composed of two groups, patients without leukocytospermia (n=115) and patients with leukocytospermia (n=70). The infertile men enrolled in the study underwent semen analysis and measurements of fructose, acid-phosphatase, zinc and γ-glutamyltranspeptidase in seminal plasma.

Results: The mean age of study participants was 33.97 ± 6.45 years. The analysis of leukocyte concentration in semen has shown that 70 (37.8%) patients had leukocytospermia. Patients with leukocytospermia had significantly decreased sperm count and vitality while other sperm parameters such as seminal volume, progressive motility, morphology of pathological forms and seminal plasma pH were not affected. The levels of acid-phosphatase, fructose and γ-glutamyltranspeptidase were significantly decreased in infertile men with leukocytospermia compared to men without this condition. The levels of seminal zinc did not differ between the two groups of patients.

Conclusion: Our results indicate that leukocytospermia has a significant negative effect on the standard semen parameters and biochemical compounds that reflect the function of accessory glands, prostate and seminal vesicles in particular.

Keywords: Sperm parameters; Seminal plasma; Male infertility; Leukocytospermia; Fructose; Zinc; Acid phosphatase; γ-glutamyltranspeptidase

Introduction

White Blood Cells (WBC) are commonly found in small quantities in semen sample in healthy men [1]. Leukocytospermia (LCS), also known as leukospermia, pyospermia or pyosemia designates abnormally high concentrations of WBC in semen [2] and is defined by the World Health Organization (WHO) in 1992 as the presence of at least 1 × 10^6 WBC per mL of semen [3]. Despite the generally accepted hypothesis that the increased number of leukocytes in body fluids indicates an infection, the presence of bacteria have been confirmed only in a minority of leukocytospermic samples [1,4,5]. Additionally, the need for antibiotic therapy for the treatment of LCS still remains controversial [6-10]. Moreover, it is important to emphasize that apart from infection other non-infectious factors such as autoimmunity, toxins, and medications can also lead to LCS [11].

Polymorphonuclear neutrophils, which represent the most prevalent type of WBC in semen, have the capacity to generate Reactive Oxygen Species (ROS) in response to a wide variety of chemical and bacterial stimuli [12,13]. The potential role of oxidative stress in the disruption of the functional competence of human spermatozoa was first recognized in 1943 by the study of McLeod [14] which showed the negative impact of high oxygen tensions on sperm motility. Subsequent studies have provided definitive evidence that human spermatozoa may generate ROS [15-17] and the increase of ROS activity was demonstrated in infertile males [18-20].

The prevalence of LCS in male infertility patients varies from 2% to 40% in published reports [21-23]. However, despite highly recognized public health burden of LCS, its clinical significance still remains unclear. Namely, numerous studies have reported that LCS is associated with abnormal standard semen parameters, sperm function, and male infertility [24,25]. In contrast, other studies have not confirmed these findings and there are reported data which show the opposite results [26]. Specifically, it was shown that leukocytes in semen could exert positive effects as they phagocytose and eliminate morphologically abnormal spermatozoa [27].

Therefore, we aimed to investigate the association of the LCS with progressive sperm motility, sperm concentrations and biochemical markers of sperm function. Determinations of biochemical constituents of seminal plasma are needed for semen evaluation in

addition to physical characteristics of semen as those biochemical compounds seem to be good predictive markers of male infertility. We evaluated the association of LCS with seminal concentration of zinc, prostate-specific acid phosphatase and γ-glutamyltranspeptidase [GGT] originating from prostate and fructose from seminal vesicles.

Materials and Methods

Patients

This cross-sectional clinical study enrolled 185 consecutive men with fertility problems referred to the Uromedica Polyclinic, Belgrade in the period from June to November 2017 who fulfilled the inclusion criteria. Inclusion criteria were: age between 20 and 39 years and written informed consent. Exclusion criteria were: presence of urogenital infection, azoospermia, antibiotic therapy during the last month. This study was approved by the Ethical Committee of the Association of Serbian Private Healthcare Providers, Belgrade, Serbia.

Semen analysis

All semen analyses were performed within 2 hrs after ejaculation, and analyzed according to the criteria as specified in the WHO Laboratory Manual 2010 [28].

Leukocytospermia determination

Leukocytes in semen were counted using procedure which identifies peroxidase enzyme present in polymorphonuclear granulocytes (PMN), the most prevalent WBC type in semen. Peroxidase was stained using ortho-toluidine as described in the WHO 2010 guideline [28]. Peroxidase in granulocytes catalyzes the reaction of ortho-toluidine and H_2O_2 and brown colored product is formed. Granulocytes stain brown as peroxidase positive cells, while peroxidase negative cells are unstained.

Briefly, working solution consisted of 10 mL 0.25% ortho-toluidine in phosphate buffer pH 6.0 mixed with 0.1 mL 30% H_2O_2. The semen sample was diluted with physiological saline solution in a ratio of 1:10 and 100 μL of diluted semen sample was mixed with 100 μL of the working solution. The mixture was incubated for 20 min at 37°C. Peroxidase positive leukocytes were counted in improved Neubauer chamber under 400x magnifications (Nikon SE, Japan).

Biochemical assays

Zinc was determined in semen with commercially available kit for measurement of serum zinc by spectrophotometric assay which was adapted for semen (Randox, ZN2341, United Kingdom) in which the compound 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropyl amino)-phenol (5-Br-PAPS) binds zinc, producing a change in colour which is measured at the wavelength 560 nm. Fructose was determined using commercially available kit (Roche-glucose HK cobas, Gluk2, Switzerland) for the quantitative, enzymatic determination of fructose in semen by Hexokinase. Fructose is phosphorylated by Adenosine Triphosphate (ATP) in the reaction catalyzed by hexokinase. Fructose 6-phosphate is converted to glucose 6-phosphate by phosphoglucoseisomerase (PGI). Glucose-6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of Nicotinamide Adenine Dinucleotide (NAD) in the reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in the absorbance at 340 nm is directly proportional to fructose concentration. Acid phosphatase was determined by Konetic colorimetric method (modified Hillman method) (Randox, AC1011, United Kingdom). γ-glutamyltranspeptidase (GGT) activity was measured by an enzymatic rate method (Clinischem Ltd, GGT-47261, Budapest, Hungary). In the reaction, the γ-glutamyltranspeptidase (GGT) catalyzes the transfer of a gamma-glutamyl group from the colorless substrate, γ-glutamyl-p-nitroaniline, with production of the colored product, p-nitroaniline.

Statistical analysis

Statistical analysis comprised calculation of descriptive measures including mean value, standard deviation, minimum and maximum value (SPSS software). The differences between groups with normal and elevated level of leukocytes have been explored by employing t test or Mann- Whitney test based on the characteristics of data distribution. A $p<0.05$ was considered as statistically significant.

Results

The mean age of study participants was 33.97 ± 6.45 years. The analysis of leukocyte concentration in semen has shown that 70 (37.8%) patients had leukocytospermia defined as number of leukocytes in semen>1.0×10^6. There was no statistically significant difference among patients with or without leukocytospermia regarding age (p=0.394).

The sperm parameters in groups of patients with and without leukocytospermia are presented in Table 1. The data obtained in this study revealed that sperm concentration was statistically significantly higher in a group of men without leukocytospermia compared with those with leukocytospermia (p=0.038). Furthermore, there was statistically significant difference regarding the percentage of vital spermatozoa between these two groups (p=0.009). Importantly, group of patients with leukocytospermia had lower vitality of spermatozoa. No statistically significant difference was detected between the study groups concerning the other investigated sperm-related characteristics (Table 1).

Sperm-related characteristics	Patients without leukocytospermia (N=115)	Patients with leukocytospermia (N=70)	P-value
Seminal volume (mL)	3.14 ± 1.59	3.22 ± 1.52	0.725
Sperm concentration (x 10^6/mL)	45.87 ± 56.26	20.93 ± 30.71	0.038
Progressive motility	32.26 ± 12.68	31.15 ± 12.94	0.531
Vitality	64.33 ± 11.55	59.28 ± 13.07	0.009
Morphology of pathological forms (%)	56.76 ± 10.28	50.59 ± 10.84	0.092
pH	7.94 ± 0.42	7.89 ± 0.34	0.452

Table 1: Sperm-related characteristics in study participants.

Seminal biochemical markers in groups of males with and without leukocytospermia are shown in (Table 2). The analysis of group-specific differences related to seminal biochemical markers has

illustrated that seminal vesicle secretion (fructose) and various prostatic markers (acid phosphatase, GGT) were statistically significantly lower in patients with leukocytospermia compared with those with normal number of white blood cells in semen. The level of zinc in semen did not differ between the patients with or without leukocytospermia (Table 2).

Seminal biochemical markers	Patients without Leukocytospermia (N=115)	Patients with Leukocytospermia (N=70)	P-value
Acid phosphatase (U/L)	1.21 ± 0.72	0.83 ± 0.53	<0.001
Fructose (mmol/L)	13.71 ± 6.80	11.80 ± 6.10	0.049
GGT (U/L)	13.29 ± 6.93	10.49 ± 4.97	0.004
Zinc (mmol/L)	2.35 ± 1.41	2.03 ± 1.41	0.08

Table 2: Seminal biochemical markers in study participants.

Discussion

The significance of genital inflammation in male infertility is unclear in many respects. Leukocytospermia has been considered as an indicator of male genital tract inflammation and it has been related to poor semen parameters [22,29-32]. The purpose of our study was to explore whether the leukocytospermia is related to seminal quality and biochemical markers of sperm function in 185 men with fertility problems. Leukocytospermia was present in 37.8% of infertile men enrolled in this study which is in accordance with previously reported incidence of this condition (2-40%) [2].

Several studies have shown the negative correlation between WBC count in semen and seminal parameters [22,33-35]. Our data confirm that leukocytospermia is associated with lower sperm concentration and sperm vitality whereas other sperm parameters such as sperm motility, pathological forms and sperm volume did not differ between patients with and without leukocytospermia. The possible explanation of these findings might be that inflammation of genital tract can cause partial obstructions of the seminal tract, leading to severe oligoasthenospermia [36]. The first demonstration that infection of the sex glands could impair their excretory function was made in late 1960s [37]. Approximately 50% of leukocytes present in the seminal fluid originate from the prostate and the seminal vesicles in the course of prostatitis or seminal vesiculitis, thus the infection of the accessory glands leads to increased number of leukocytes in the ejaculate.

In this study we measured the biochemical compounds in semen derived from accessory glands in order to assess gland function including zinc, γ-glutamyltranspeptidase (GGT) and acid phosphatase for the prostate and fructose for the seminal vesicles function. The obtained results show that men with fertility problems and leukocytospermia have significantly decreased levels of acid-phosphatase, GGT and fructose compared to those without leukocytospermia. The levels of zinc was also decreased in patients with abnormally high PMN count in semen but without reaching statistical significance which is in agreement with the reported data [38].

Inflammatory conditions considerably influence the secretory function of the male accessory organs. Decreased levels of citric acid, acid phosphatase, fructose, zinc and alpha-glutamyltransferase activity have been shown to be associated with decreased prostatic and seminal vesicle secretory function [39-44]. Marconi et al. [45] demonstrated decreased seminal fructose level in urogenital infection, but concluded that its measurement cannot discriminate patients with and without infection. Wolf et al. [41] demonstrated negative correlation between levels of fructose and PMN elastase in semen. This is in accordance with our results of significantly lower seminal fructose level in infertile men with leukocytospermia. Bezold et al. [46] showed that fructose levels in seminal plasma were not decreased in a population of infertile men with sexually transmitted disease.

The levels of citric acid, γ-glutamyltranspeptidase (GGT) and zinc in semen and seminal plasma pH have been proposed as biochemical markers of prostate gland exocrine function, which concentrations are altered during bacterial infection and inflammation. The concentration of γ-glutamyltransferase is approximately 200 times higher in the semen than in the serum [47]. The role of GGT in male fertility is not clear, and the results from different studies are conflicting [47,48]. Recent studies showed that GGT plays a major role in the glutathione system and formation of cysteine that protects spermatozoa against oxidative stress [49,50]. In our study infertile men with leukocytospermia had significantly lower GGT level in seminal plasma and this finding is in accordance with the reported data of positive correlation of this enzyme with sperm count showing significantly lower level of GGT in a group of oligospermic men compared to a group of men with normospermia and asthenospermia [51,52]. Similarly, Marconi et al. [45] found the significant decrease of the level of GGT in patients with inflammatory chronic prostatitis/chronic pelvic pain syndrome, but they concluded that GGT could not be used as a diagnostic marker of inflammation in chronic prostatitis/chronic pelvic pain syndrome due to low sensitivity and specificity.

Zinc has been suggested to contribute to bactericidal activity of human seminal plasma [53]. Moreover, prostatic secretions contain the highest physiologic concentrations of zinc in men; decades ago, zinc was identified as the "*prostatic antibacterial factor*" [54].

In conclusion, the clinical significance of elevated number of WBC in the male ejaculate is still controversial. Evidence suggests that silent inflammation of the genital tract can deteriorate the fertile potential of men. The origin of infertility in men with the leukocytospermia may be the presence of subclinical genital inflammation which can lead to deterioration of the spermatogenesis, impairment of sperm function, and obstruction of the seminal tract [55,56]. Further investigations are needed to confirm these hypotheses.

The data obtained in this study clearly indicate that leukocytospermia in infertile men is associated with impairment of secretory function of prostate and seminal vesicles as evidenced in decreased levels of biochemical compounds derived from these glands in seminal plasma. In addition, leukocytospermia in infertile men related to the abnormalities of certain parameters of spermogram such as sperm concentration and vitality.

References

1. Wolff H (1995) The biologic significance of white blood cells in men. Fertil Steril 63: 1143-1157.
2. Centola GM, Ginsburg KA (1996) Evaluation and treatment of the infertile male. Cambridge University Press pp. 263-276.
3. World Health Organization (1999) WHO laboratory manual for the examination of human semen and sperm-cervical interaction. Cambridge: Cambridge University Press.

4. Cottell E, Harrison RF, McCaffrey M, Walsh T, Mallon E, et al. (2000) Are seminal fluid microorganisms of significance or merely contaminants? Fertil Steril 74: 465-470.
5. Trum JW, Mol BW, Pannekoek Y, Spanjaard L, Wertheim P, et al. (1998) Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. FertilSteril 70: 315-319.
6. Erel CT, Senturk LM, Demir F, Irez T, Ertungealp E (1997) Antibiotic therapy in men with leukocytospermia. Int J Fertil Womens Med 42: 206-210.
7. Branigan EF, Spadoni LR, Muller CH (1995) Identification and treatment of leukocytospermia in couples with unexplained infertility. J Reprod Med 40: 625-629.
8. Yanushpolsky EH, Politch JA, Hill JA, Anderson DJ (1995) Antibiotic therapy and leukocytospermia: A prospective, randomized, controlled study. Fertil Steril 63: 142-147.
9. Zorn B, Virant-Klun I, Meden-Vrtovec H (2000) Semen granulocyte elastase: Its relevance for the diagnosis and prognosis of silent genital tract inflammation. Hum Reprod 15: 1978-1984.
10. Branigan EF, Muller CH (1994) Efficacy of treatment and recurrence rate of leukocytospermia in infertile men with prostatitis. Fertil Steril 62: 580-584.
11. Menkveld R (2004) Leukocytospermia: Advances in fertility and reproductive medicine. International Congress Series No. 1266 pp. 218-224.
12. Aitken RJ, Fisher HM, Fulton N, Gomez E, Knox W, et al. (1997) Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavoprotein inhibitors diphenyleneiodonium and quinacrine. Mol Reprod Dev 47: 468-482.
13. Krausz C, Mills C, Rogers S, Tan SL, Aitken RJ (1994) Stimulation of oxidant generation by human sperm suspensions using phorbol esters and formyl peptides: Relationships with motility and fertilization in vitro. Fertil Steril 62: 599-605.
14. MacLeod J (1943) The role of oxygen in the metabolism and motility of human spermatozoa. Am J Physiol 138: 512-518.
15. Aitken RJ, Clarkson JS (1988) Significance of reactive oxygen species and anti-oxidants in defining the efficacy of sperm preparation techniques. J Androl 9: 367-376.
16. Alvarez JG, Touchstone JC, Blasco L, Storey BT (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8: 338-348.
17. Iwasaki A, Gagnon C (1992) Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 57: 409-416.
18. Aitken RJ, Clarkson JS, Hargreave TB, Irvine DS, Wu FCW (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.
19. Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K, et al. Differential contribution of leukocytes and spermatozoa to the high levels of reactive oxygen species recorded in the ejaculates of oligozoospermic patients. J Reprod Fertil 94: 451-462.
20. Sharma RK, Agarwal A (1996) Role of reactive oxygen species in male infertility. Urology 48: 835-850.
21. Krause W, Herbstreit F, Slenzka W (2002) Are viral infections the cause of leukocytospermia? Andrologia 34: 87-90.
22. Wolff H, Politch JA, Martinez A, Haimovici F, Hill JA, et al. (1990) Leukocytospermia is associated with poor semen quality. Fertil Steril 53: 528-536.
23. Arata de Bellabarba G, Tortolero I, Villarroel V, Molina CZ, Bellabarba C, et al. (2000) Non sperm cells in human Semen and their relationship with semen parameters. Arch Androl 45: 131-136.
24. Fedder J (1996) Non sperm cells in human semen: With special reference to seminal leukocytes and their possible influence on fertility. Arch Androl 36: 41-65.
25. Aitken RJ, Clarkson JS, Fishel S (1989) Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod 41: 183-197.
26. Kiessling AA, Lamparelli N, Yin HZ, Seibel MM, Eyre RC (1995) Semen leukocytes: Friends or foes? FertilSteril 64: 196-198.
27. Tomlinson MJ, White A, Barratt CL, Bolton AE, Cooke ID (1992) The removal of morphologically abnormal sperm forms by phagocytes: A positive role for seminal leukocytes? Hum Reprod 7: 517-522.
28. WHO (2010) WHO laboratory manual for the examination and processing of human semen. 5th Edition, Cambridge: Cambridge University Press.
29. Zorn B, Virant-Klun I, Meden-Vrtovec H (2000) Semen granulocyte elastase: Its relevance for the diagnosis and prognosis of silent genital tract inflammation. Hum Reprod 15: 1978-1984.
30. Aitken RJ, Gordon-Baker HW (1995) Andrology: Seminal leukocytes: Passengers, terrorists or good Samaritans? Hum Reprod 10: 1736-1739.
31. Rajasekaran M, Hellstrom WJG, Naz R, Sikka SG (1995) Oxidative stress and interleukins in seminal plasma during leukocytospermia. Fertil Steril 64: 166-171.
32. Yanushpolsky EH, Politch JA, Hill JA, Anderson DJ (1996) Is leukocytospermia clinically relevant? Fertil Steril 66: 822-825.
33. Perez-Pelaez M, Jeyendran RS, Soberero AJ (1982) Correlation between semen analysis results and physical examination in sub fertile men. J Androl 3: 36.
34. Thomas J, Fishel SB, Hall JA, Green S, Newton TA, et al. (1997) Increased polymorphonuclear granulocytes in seminal plasma in relation to sperm morphology. Hum Reprod 12: 2418-2421.
35. Van der Ven HH, Jeyendran RS, Perez-Pelaez M, Al Hasini S, Diedrich K, et al. Leukospermia and the fertilizing capacity of spermatozoa. Eur J Obstet Gynecol Reprod Biol 24: 49-52.
36. Keeck C, Gerber SC, Clad A, Wilhelm C, Breckwoldt M (1998) Seminal tract infections impact on male fertility and treatment options. Hum Reprod 4: 891-903.
37. Eliasson R (1968) Biochemical analysis of human semen in the study of the physiology and pathophysiology of the male accessory sex glands. Fertil Steril 19: 344-350.
38. Eggber KW, Zwick EM, Batshulat MD, Rohr G, Ambruster FP, et al. (2002) Are zink levels in seminal plasma associated with seminal leukocytes and other determinants of semen quality. FertilSteril 77: 260-269.
39. Weidner W, Krause W, Schiefer HG, Brunner H, Friedrich HJ (1985) Urea plasma infections of the male urogenital tract, in particular, prostatitis, and semen quality. Urol Int 40: 5-9.
40. Cooper TG, Weidner W, Nieschlag E (1990) The influence of inflammation of the human genital tract on secretion of the seminal markers alpha glucosidase, glyceryl- phosphocholine, carnitine, fructose and citric acid. Int J Androl 13: 329-336.
41. 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 132: 331-334.
42. Purvis K, Christansen E (1995) The impact of infection in sperm quality. J Br Fertil Soc 1: 31-41.
43. Depuydt CE, Bosmans E, Zalata A, Schoonjans F, Comhaire FH (1996). The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. J Androl 17: 699-707.
44. Comhaire FH, Verschraegen G, Vermeulen L (1980) Diagnosis of accessory gland infection and its possible role in male infertility. Int J Androl 3: 32-45.
45. Marconi M, Pilatz A, Wagenlehner F, Diemer T, Weidner W (2009). Impact of infection on the secretory capacity of the male accessory glands. Int J Brazil Urol 35: 299-309.
46. Bezold G, Politch JA, Kiviat NB, Kuypers JM, Wolff H, et al. (2007) Prevalence of sexually transmissible pathogens in semen from

asymptomatic male infertility patients with and without leukocytospermia. Fertil Steril 87: 1087-1097.

47. Uchijima Y, Yoshida K, Hiraga S (1986) Studies on Gamma-glutamyl transpeptidase (gamma-GTP) in seminal plasma. Hinyokika Kiyo 32: 353-359.
48. Aafjes JH, Wolffensperger-Van Oort HJ, Schenck PE (1983) Gamma-glutamyltransferase and beta-glucuronidase in human ejaculate. Relation with abnormal liquefaction. Andrologia 15: 661-665.
49. Yokoyama H (2007) Gamma-glutamyl transpeptidase (gamma-GTP) in the era of metabolic syndrome. Nihon Arukoru YakubutsuIgakkai Zasshi 42:110-124.
50. Pero ME, Lombardi P, Longobardi V, Boccia L, Vassaloti G, et al. (2017) Influence of gamma glutamyl transferase and alkaline phosphatase activity on in vitro fertilization of bovine frozen/thawed semen. Ital J Anim Sci 16: 390-392.
51. Chen F, Lu JC, Xu HR, Huang YF, Hu YA, et al. (2006) Evaluation of the determination of seminal acid phosphatase (ACP) and gamma- glutamyl transferase (gamma-GT) activities and correlation between seminal ACP or gamma-GT activity and semen parameters. Zhongua Nan KeXue 12: 879-882.
52. Awadallah SM, Salem N, Saleh SA, Mubarak MS, Elkarmi AZ (2003) Zinc, magnesium and gamma glutamyltransferase levels in human seminal fluid. Bahrein Med Bull 23: 1-7.
53. Edström AM, Malm J, Frohm B, Martellini JA, Giwercman A, et al. (2008) The major bactericidal activity of human seminal plasma is zinc-dependent and derived from fragmentation of the semenogelins. J Immunol 181: 3413-3421.
54. Fair WR, Couch J, Wehner N (1976) Prostatic antibacterial factor: identity and significance. Urology 7: 169-177.
55. Bar-Chama N, Fisch H (1993) Infection and pyospermia in male infertility. World J Urol 11: 76-81.
56. Purvis K, Christansen E (1996) The impact of infection on sperm quality. Hum Reprod 11: 31-41.