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The effect of varicocoelectomy on the relationship of oxidative stress in peripheral and internal spermatic vein with semen parameters

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SUMMARY

The aim of this prospective controlled study was to investigate the levels of reactive oxygen species (ROS), including asymmetric dimethylarginine (ADMA), oxidative stress index (OSI) and total oxidant capacity (TOC), and antioxidants with total antioxidant capacity (TAC) in peripheral and internal spermatic veins blood, the relationship of these factors with sperm parameters in the infertile varicocoele patients, and the amelioration effect of varicocoelectomy on these outcomes. Thirty-one primary infertile varicocoele patients and 31 fertile control patients evaluated for determining the levels of ADMA, TOC, OSI, superoxide dismutase (SOD), glutathione (GSH), TAC, and semen analysis. The patients' preoperative SOD, GSH and TAC levels, which were significantly lower than the controls, significantly increased postoperatively. Although SOD and GSH were significantly higher in spermatic vein compared to median cubital vein, TAC was significantly higher in median cubital vein. ADMA, TOC and OSI were significantly higher in the patient group. TOC and OSI were significantly higher in spermatic vein compared to median cubital vein. Postoperative TOC, OSI and ADMA reduced to the control levels. Total antioxidant capacity in the peripheral circulation and oxidative stress index in the internal spermatic vein could give an idea about the possible improvement in sperm count acquired by varicocoelectomy.

INTRODUCTION

Infertility is defined by World Health Organization (WHO) as the inability to achieve spontaneous pregnancy within 1 year for sexually active non-contracepting couples (WHO, 2000). Male infertility associated with abnormal semen parameters is the reason in about 50% of the infertile couples (Sanocka & Kurpisz, 2003). Varicocoele, which presents in 11.7% of adult men and in 25.4% of men with abnormal semen characteristics, is one of the main male-related infertility factors (WHO, 1992). Although the pathophysiology of varicocoele is not known precisely, there are some hypotheses such as germ cell dysfunction, decreased secretion of gonadotropins and androgens, retrograde flow of metabolites, testicular hypoxia, oxidative stress, and increased temperature to explain the resultant infertility.

The relationship between varicocoele and oxidative stress because of the overproduction of reactive oxygen species (ROS) and a decrease in the antioxidant level, has been studied in many previous studies (Hendin *et al.*, 1999; Pasqualotto *et al.*, 2000; Ozbek *et al.*, 2008) and still has a mysterious side. ROS can easily damage spermatozoa because of high level of unsaturated fatty acids in their cell membrane and low level of enzymatic antioxidants in their cytoplasm, and can eventually cause the decrease in sperm count and motility, and the increase in abnormality of sperm morphology (Henkel & Schill, 1998; Pasqualotto *et al.*, 2000; Sanocka-Maciejewska *et al.*, 2005). On the other hand, spermatozoa and seminal plasma have a protective system, including enzymatic and non-enzymatic antioxidants, and low molecular weighted compounds with scavenger feature, to prevent oxidative stress via neutralizing and removing ROS (Walczak-Jedrzejowska *et al.*, 2013).

Asymmetric dimethylarginine (ADMA), formed via proteolysis of methylated arginine residues, is a competitive inhibitor of nitric oxide synthase (NOS), which facilitates the formation of nitric oxide (NO) and citrulline from arginine (Visser *et al.*, 2010). ADMA, a new parameter became popular in the assessment of oxidative stress, causes the decrease in NO level by NOS inhibition and the increase in ROS by NOS uncoupling, and can eventually induce oxidative stress, endothelial dysfunction and

impaired anti-thrombotic and anti-inflammatory activities (Visser *et al.*, 2010; Aldámiz-Echevarría & Andrade, 2012).

In this study, to obtain a predictive information about fertility status after varicocoelectomy via systemic blood analysis, we explored the levels of ROS including ADMA and antioxidants in the peripheral and the internal spermatic veins blood of the patients with varicocoele, the relationship of these factors and sperm parameters, and the amelioration effect of varicocoelectomy on these outcomes comparing with a control group.

MATERIALS AND METHODS

In this prospective study, 31 patients with grade 2 and 3 unilateral left varicocoele disease and primary infertility prolonged more than 2 years and 31 fertile nonsmoker control patients attended to our urology department for any other reason, such as lombar pain and urolithiasis, except infertility were evaluated. This study, conducted in accordance with Declaration of Helsinki, was approved by Ethics Committee of Clinical Research in Malatya (protocol number: 2013/64). Physical examination, scrotal color doppler ultrasonography to measure the size of pampiniform venous plexus, hormonal assessment and semen analysis performed according to the guidelines of World Health Organization were used for evaluation in both patients and control groups. The patients with other testicular and pre-testicular diseases causing infertility except varicocoele and some specific conditions such as genitourinary tract infection, diabetes, and smoking, which may lead to changes in oxidative stress, were excluded. The patients and controls with leukospermia (10^6 leukocytes/mL) in their semen analysis were not included on account of the negative effect of infection on oxidative stress.

Oxidative stress status were evaluated in the peripheral blood samples drawn from the median cubital vein in both of the control group and the patient group before and 3 months after the surgery and the internal spermatic vein during inguinal approached left varicocoelectomy surgery in the patient group only. Approximately 5 cc blood was carefully drawn from internal spermatic vein just before the any manipulation and ligation of dilated veins according to the previously reported method (Mostafa *et al.*, 2006). After centrifuging the blood specimens, the plasma samples were kept at −80 °C till further analysis for determining the levels of ADMA, total oxidant capacity (TOC) and the oxidative stress index (OSI) as the oxidative parameters, and superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity (TAC) as the antioxidant parameters.

Semen specimens were ideally collected by masturbation on the fourth day of abstinence from both the control group and the patient group before and 3 months after varicocoelectomy and analysis was carried out according to the guidelines of WHO Laboratory Manual for the Examination and Processing of Human Semen (WHO, 2010).

Biochemical analyses

SOD: Total SOD activity was determined by the method of Sun *et al.* (Sun *et al.*, 1988). The principle of this method is the inhibition of nitroblue tetrazolium reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was measured as the enzyme amount inducing 50% inhibition in the nitroblue tetrazolium reduction rate. SOD activity was shown as U/gm protein.

GSH: GSH was measured by spectrophotometry by the method of Ellman (1959). Each sample was mixed with 10 mM 5,5′-dithiobis (2-nitrobenzoic acid) in 100 mM potassium phosphate buffer (pH 7.5) and 17.5 M ethylenediaminetetraacetic acid. The reaction was started by adding 0.5 U GSH reductase and 0.4 mM NADPH. At 5 min absorbance was determined at 410 nm. The GSH concentration was measured against a standard curve. The results were shown as mmol/gm.

TAC: TAC was determined by spectrophotometry using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) (Erel, 2004). The method is based on the disappearance/fading of the dark blue-green color of the stable ABTS (2,2′-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid) radical in proportion to the amount of antioxidants in the environment. The disappearance of this color increases in parallel to the amount of antioxidants in the environment. Calculations were made according to the standard kit. The results were shown as mmol Trolox™ equivalents per L.

TOC: TOC was assessed by spectrophotometry using commercial kits (Rel Assay Diagnostics) (Erel, 2005). This new method is based on the oxidation of ferrous ion-o-dianisidine complexes into ferric ions by oxidant compounds in the samples. Spectrophotometric measurements were carried out according to the color density obtained. This measurement indicated the total amount of oxidant molecules in the environment. The results were shown as mmol H_2O_2 equivalents per L.

OSI: OSI is the ratio of TOC in mmol H_2O_2 equivalents per L to TAC in mmol Trolox equivalents per L (Harma & Erel, 2003).

ADMA: A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit was used to determine the amount of ADMA in the blood samples (Schulze *et al.*, 2004). The principle of this method is the estimate of ADMA bound to microtiter plate using rabbit anti-ADMA antiserum. The intensity of substrate/peroxidase reaction was monitored at 450 nm. The result was given as ng per L.

Statistical analysis

IBM SPSS statistics version 22.0 for Windows was used for statistical analyses. The data were expressed as median (min - max) or mean ± SD values depending upon overall variable distribution. Normality was assessed using Shapiro Wilk test. The groups were compared by Mann–Whitney *U* test or independent samples *t* test. The non-normally distributed data for repeated observations were compared by Friedman test among the groups. When significant differences were determined, multiple comparisons were carried out using Wilcoxon test with Bonferroni correction. Correlations were estimated using Spearman's rho as appropriate. $p < 0.05$ values were considered as significant.

RESULTS

The mean age was 28.7 ± 4.48 (range 21–36 years) and 29 ± 4.18 (range 22–36 years) in the patient and control groups, respectively, and there was no significant difference. As shown in Table 1, the levels of SOD and GSH, and TAC were significantly higher in the blood of control group when compared to the blood of peripheral and internal spermatic veins of patient group. On the other hand, SOD and GSH levels were significantly higher in spermatic vein blood when compared to median cubital vein blood in the patient group; however, TAC was

Table 1 The levels of SOD, GSH, TAC, TOC, OSI, and ADMA in the blood samples of the control and the patient groups

	Control Group M.Cubital Vein	Patient Group		
		Preop. M.Cubital Vein	I. Spermatic Vein	Postop. M.Cubital Vein
SOD	28.6 (24–38)[a]	15 (11–17.7)[b]	21.6 (17.2–27.3)[c]	27.3 (24.2–34.4)[a]
GSH	357.6 (210.2–402.5)[a]	193.5 (164–238.4)[b]	235.8 (182–277)[c]	316.3 (286.4–371.7)[d]
TAC	0.51 (0.43–0.74)[a]	0.47 (0.26–0.69)[b]	0.4 (0.22–0.52)[c]	0.52 (0.41–0.78)[a]
TOC	2.28 (1.43–3.89)[a]	3.47 (2.02–6.77)[b]	4.5 (2.03–8.99)[c]	2.45 (1.16–3.75)[a, b]
OSI	4.65 (2.65–7.53)[a]	7.7 (3.6–16.1)[b]	10.6 (5.1–39)[c]	4.6 (1.7–7.7)[a]
ADMA	10.88 (9.3–15)[a]	20.6 (16.5–28)[b]	19.1 (15.5–26.5)[b]	11.5 (8.3–15.4)[a]

All results were given as median (min-max). $p < 0.05$ statistically significant in all assessments. Different letters written as superscript mean the significant difference in comparison of each other in each row.

significantly higher in median cubital vein blood. ADMA level, TOC and OSI were significantly higher in the patient group when compared to the control group. Although TOC and OSI were significantly higher in spermatic vein blood when compared to median cubital vein blood, but there was no significant difference in ADMA level between them. In addition, as shown in Table 2, semen parameters, including concentration and progressive motility, were significantly lower in the patient group preoperatively when compared to the control group.

The postoperative levels of SOD, GSH, and TAC in the patient group were significantly higher when compared to the preoperative results. Moreover, the preoperative reduced level of SOD and TAC were nearly increased to the control levels. On the other hand, postoperative TOC, OSI and ADMA level reduced to the control levels; however, there was no significant difference found between pre- and postoperative TOC level in median cubital vein blood of the patient group. Postoperative sperm parameters were significantly increased, not to the control levels, when compared to the preoperative results.

In correlation analysis, there were a positive significant relationship between OSI in the internal spermatic vein blood and postoperative sperm count (Spearman's rho: 0.65; $p < 0.05$), and a negative significant relationship between TAC in the median cubital vein blood and postoperative sperm count (Spearman's rho: 0.51; $p < 0.05$). On the other hand, a positive significant relationship was found between ADMA and progressive sperm motility in the control group (Spearman's rho: 0.42; $p < 0.05$).

DISCUSSION

Varicocoele is a disease causing semen abnormalities and one of the surgically treatable reasons of male infertility. The exact mechanism between varicocoele and male infertility is still not clear; however, testicular hypoxia, renal and adrenal metabolites back-flow, and high scrotal temperature are mostly reported reasons (Chen *et al.*, 2004). Recently, DNA damage of spermatozoa, which has been shown to be related with varicocoele disease, was reported to be secondary to increase in ROS and could be diminished by varicocoelectomy (Zini & Dohle, 2011). Increase in ROS level in seminal fluid was determined in the infertile male patients with varicocoele disease (Hendin *et al.*, 1999). In a previous study, it was reported that varicocoele caused a rise in ROS level and a reduction in the antioxidant capacity in seminal fluid (Mostafa *et al.*, 2001). The overproduction of ROS and a decrease in the level of antioxidants give rise to oxidative stress, which has damaging influence on spermatozoa (Altintas *et al.*, 2012). On the other hand, the positive effect of ROS on capacitation, hyperactivation, and acrosome reaction of spermatozoa has been revealed (Zalata *et al.*, 2004).

Table 2 The sperm concentration and motility in the control and patient groups

	Control Group	Patient Group	
		Preoperative	Postoperative
Sperm concentration (10^6/mL)	65 (35–145)[a]	11 (5–14)[b]	20 (5–52)[c]
Motility (%)	50 (40–90)[a]	20 (0–60)[b]	30 (0–80)[c]

All results were given as median (min-max). $p < 0.05$ statistically significant in all assessments. Different letters written as superscript mean the significant difference in comparison of each other in each row.

Increased level of ROS was shown in 40% of the infertile patients; however, this ratio was 80% in the infertile patients with varicocoele (Lewis *et al.*, 1995). Moreover, the reduction in antioxidant level was determined in both of seminal fluid and blood of the patients with varicocoele disease (Barbieri *et al.*, 1999). In the same study, the local and systemic existences of oxidative stress were also reported. In this study, oxidative stress markers were significantly higher in varicocoele patients than the control group. Moreover, TOC and OSI were significantly higher in the internal spermatic vein when compared with the peripheral circulation. In a previous study, Mostafa *et al.* (2006) reported a significantly increase in ROS level in the internal spermatic vein compared with the peripheral venous circulation in the infertile patients with varicocoele.

Asymmetric dimethylarginine, a metabolite of arginine metabolism, can be used as a cardiovascular risk factor and contribute to many deleterious events such as cardiac remodeling, fibrosis, angiogenesis, endothelial dysfunction, and myocardial ischemia (Visser *et al.*, 2010). Also, ADMA can uncouple NOS that results in the formation of superoxide radicals (Suda *et al.*, 2005). Moreover, low-density lipoproteins and oxidized low-density lipoproteins can induce further ADMA formation in oxidative stress (Böger *et al.*, 2000). In this study, although ADMA level was significantly higher in the patient group than the control group, there was no significant difference between the blood samples of the internal spermatic vein and the peripheral circulation in the patient group. On the other hand, Kiziler *et al.* reported that ADMA level in the internal spermatic vein was lower than in the peripheral circulation (Kiziler *et al.*, 2015).

In our study, the examined antioxidants, SOD and GSH, were found to be significantly higher in the internal spermatic vein than in the median cubital vein in the patient group, however, these are significantly lower than in the control group. Superoxide dismutase and GSH are important parts of the defense mechanism of organism and testis against ROS and lipid peroxidation,

but spermatozoa don't have GSH (Bauché *et al.*, 1994). In parallel to our results, Ozbek *et al.* (2008) found that the levels of the investigated antioxidants, SOD and GSH, were significantly higher in the internal spermatic vein than in the peripheral circulation, and explained these increases as a reflex of organism to offset oxidative stress. Whereas, in another study, the same antioxidants were significantly lower in the internal spermatic vein compared to the peripheral circulation (Mostafa *et al.*, 2006). Another result of our study was the significantly lower value of TAC in the patient group compared to the control group, moreover, it was significantly lower in the internal spermatic vein than in the peripheral circulation. In our opinion this outcome can be found because of the exhaustion of antioxidant to bring oxidative stress under control. Barbieri *et al.* (1999) also reported the decrease in the total antioxidant defenses of both seminal fluid and peripheral circulation in varicocoele patients.

In the previous studies, it has been reported that ROS decreased in the seminal fluid and peripheral circulation after varicocoelectomy (Mostafa *et al.*, 2001; Kiziler *et al.*, 2015). According to our results, varicocoelectomy significantly reduced the examined oxidative stress parameters, TOC and ADMA, even to the control levels. Although OSI decreased to the control level, this reduction was not statistically significant compared to the preoperative one. Contrarily, in the study by Kiziler *et al.* (2015), the influence of varicocoelectomy on oxidative stress was evaluated in 29 consecutive infertile patients associated with varicocoele, and they found that varicocoelectomy increased ADMA level. But, in that study, the blood samples for the postoperative control were obtained from the femoral vein instead of the internal spermatic vein. On the other hand, varicocoelectomy significantly increased the tested antioxidants levels; moreover, SOD and TAC values increased to the control levels in our study. The increase in these antioxidant levels may be indirectly because of a decrease in exhaustion of them resulting from less formation of oxidants after varicocolectomy. Although there is no previous study comparing preoperative and postoperative oxidant and antioxidant, tested in our study, levels of blood sample in the infertile varicocoele patients with a healthy control group, there are many studies related on the favorable effect of varicocoelectomy on these parameters in seminal fluid (Mostafa *et al.*, 2001; Mancini *et al.*, 2004). In a controlled study on the infertile varicocoele patients with varicocoele reported by Ozturk *et al.* (2012), it was concluded that although varicocoelectomy ameliorated TAC value in seminal fluid, the improvement was significant in the patients with grade II and III varicocoele.

In addition, we found a negatively relationship of the preoperative TAC value in the peripheral circulation and a positive relationship of OSI in the internal spermatic vein with the sperm count obtained in the varicocoele patients postoperatively. Another interesting result of our study was a positive weak correlation between ADMA in peripheral circulation and progressive sperm motility in the control group. While the favorable effect of limited amount of ROS on sperm capacitation, acrosome reaction and motility have been reported by Griveau & de Lannou (1997); we did not find any literature related on the correlation between the investigated parameters in our study and the postoperative sperm parameters. Beside such positive effect of limited amount of oxidants on sperm capacitation and motility, the hazardous effect of ADMA has been shown under oxidative stress circumstances rather than normal condition (Busch *et al.*, 2006). The positive relationship between TAC value of seminal fluid and sperm parameters was evaluated in many previous studies (Barbieri *et al.*, 1999; Ozturk *et al.*, 2012). Moreover, the increase in ROS in seminal fluid and the decrease in semen parameters were reported to be parallel to varicocoele grade (Cocuzza *et al.*, 2012). In this study, the low level of TAC and the high level of OSI in the varicocoele patients showed us the severity of oxidative stress and the correlation between these parameters and the increase in the postoperative sperm count demonstrated the detrimental effect of varicocoele induced oxidative stress on sperm count and motility, and the improvement effect of varicocolectomy on this impaired semen quality.

CONCLUSION

This study confirms the harmful effect of varicocoele induced oxidative stress in the local and the systemic circulation on sperm parameters and the improvement effect of varicocoelectomy in the infertile patients as emphasized in the previous studies. The blood sampling for TAC in the peripheral circulation and OSI in the internal spermatic vein could give an idea about the possible improvement in sperm count acquired by varicocoelectomy.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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REFERENCES

Aldámiz-Echevarría L & Andrade F. (2012) Asymmetric dimethylarginine, endothelial dysfunction and renal disease. *Int J Mol Sci* 13, 11288–11311.

Altintas R, Parlakpinar H, Beytur A, Vardi N, Polat A, Sagir M & Odabas GP. (2012) Protective effect of dexpanthenol on ischemia-reperfusion-induced renal injury in rats. *Kidney Blood Press Res* 36, 220–230.

Barbieri ER, Hidalgo ME, Venegas A, Smith R & Lissi EA. (1999) Varicocele-associated decrease in antioxidant defenses. *J Androl* 20, 713–717.

Bauché F, Fouchard MH & Jégou B. (1994) Antioxidant system in rat testicular cells. *FEBS Lett* 349, 392–396.

Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D & Bode-Boger SM. (2000) LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine dependent methyltransferases. *Circ Res* 87, 99–105.

Busch M, Fleck C, Wolf G & Stein G. (2006) Asymmetrical (ADMA) and symmetrical dimethylarginine (SDMA) as potential risk factors for cardiovascular and renal outcome in chronic kidney disease - possible candidates for paradoxical epidemiology? *Amino Acids* 30, 225–232.

Chen CH, Lee SS, Chen DC, Chien HH, Chen IC, Chu YN, Liu JY, Chen WH & Wu GJ. (2004) Apoptosis and kinematics of ejaculated spermatozoa in patients with varicocele. *J Androl* 25, 348–353.

Cocuzza M, Athayde KS, Alvarenga C, Srougi M & Hallak J. (2012) Grade 3 varicocele in fertile men: a different entity. *J Urol* 187, 1363–1368.

Ellman GL. (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* 82, 70.

Erel O. (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 37, 277.

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 38, 1103.

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

Harma M & Erel O (2003) Increased oxidative stress in patients with hydatidiform mole. *Swiss Med Wkly* 133, 563.

Hendin BN, Kolettis PN, Sharma RK, Thomas AJ Jr & Agarwal A. (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol* 161, 1831–1834.

Henkel R & Schill WB. (1998) Sperm separation in patients with urogenital infections. *Andrologia* 30, 91–97.

Kiziler AR, Aydemir B, Guzel S, Yazici CM, Gulyasar T, Malkoc E & Acar A (2015) Comparison of before and after varicocelectomy levels of trace elements, nitric oxide, asymmetric dimethylarginine and malondialdehyde in the seminal plasma and peripheral and spermatic veins. *Biol Trace Elem Res* 167, 172–178.

Lewis SE, Boyle PM, McKinney KA, Young IS & Thompson W. (1995) Total antioxidant capacity of seminal plasma is different in fertile and infertile men. *Fertil Steril* 64, 868–870.

Mancini A, Meucci E, Milardi D, Giacchi E, Bianchi A, Pantano AL, Mordente A, Martorana GE & de Marinis L. (2004) Seminal antioxidant capacity in pre- and postoperative varicocele. *J Androl* 25, 44–49.

Mostafa T, Anis TH, El-Nashar A, Imam H & Othman IA. (2001) Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl* 24, 261–265.

Mostafa T, Anis TH, Ghazi S, El-Nashar AR, Imam H & Osman IA. (2006) Reactive oxygen species and antioxidants relationship in the internal spermatic vein blood of infertile men with varicocele. *Asian J Androl* 8, 451–454.

Ozbek E, Cekmen M, Simsek A, Turkoz Y, Soylu A, Ilbey YO & Balbay MD. (2008) Comparison of antioxidant enzyme activity in the internal spermatic vein and brachial veins of patients with infertile varicocele. *Int Urol Nephrol* 40, 679–683.

Ozturk U, Ozdemir E, Buyukkagnici U, Dede O, Sucak A, Celen S & Imamoglu MA. (2012) Effect of spermatic vein ligation on seminal total antioxidant capacity in terms of varicocele grading. *Andrologia* 44, 199–204.

Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ & Agarwal A. (2000) Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril* 73, 459–464.

Sanocka D & Kurpisz M. (2003) Infertility in Poland–present status, reasons and prognosis as a reflection of Central and Eastern Europe problems with reproduction. *Med Sci Monit* 9, 16–20.

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

Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP & Böger RH. (2004) Determination of ADMA using a novel ELISA assay. *Clin Chem Lab Med* 42, 1377–1383.

Suda O, Smith LA, d'Uscio LV, Peterson TE & Katusic ZS (2005) In vivo expression of recombinant vascular endothelial growth factor in rabbit carotid artery increases production of superoxide anion. *Arterioscler Thromb Vasc Biol* 25, 506–511.

Sun Y, Oberley L & Li Y (1988) A simple method for clinical assay of superoxide dismutase. *Clin Chem* 34, 497.

Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisselink W, de Mol BA & van Leeuwen PA. (2010) The role of asymmetric dimethylarginine and arginine in the failing heart and its vasculature. *Eur J Heart Fail* 12, 1274–1281.

Walczak-Jedrzejowska R, Wolski JK & Slowikowska-Hilczer J. (2013) The role of oxidative stress and antioxidants in male fertility. *Cent European J Urol* 66, 60–67.

World Health Organisation. (1992) The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril* 57, 1289–1293.

World Health Organization. (2000) *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple*. Cambridge University Press, Cambridge.

World Health Organization (2010) *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 5th edition. WHO Press, Switzerland.

Zalata AA, Ahmed AH, Allamaneni SS, Comhaire FH & Agarwal A. (2004) Relationship between acrosin activity of human spermatozoa and oxidative stress. *Asian J Androl* 6, 313–318.

Zini A & Dohle G. (2011) Are varicoceles associated with increased deoxyribonucleic acid fragmentation? *Fertil Steril* 96, 1283–1287.

