

Oxidative/Antioxidant Balance in Male Infertility: A Comparison of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Protein Carbonyl Levels, and Total Sulfhydryl Levels

Recep Eryılmaz¹, Muhammed Fatih Keleş¹, Rahmi Aslan¹, Veli Duman^{1*}, Murat Demir¹, Kasım Ertaş², Kerem Taken¹

¹Department of Urology, Yüzüncü Yıl University, Van, Turkey

²Department of Urology, Private Akdamar Hospital, Van, Turkey

ABSTRACT

Oxidative imbalance is recognized as a key factor in male infertility. This study aimed to compare systemic redox markers, including total oxidant status (TOS), total antioxidant status (TAS), protein carbonyl levels (PCL), and total sulfhydryl (T-SH) levels, between infertile men and healthy controls.

This controlled clinical research involved 40 infertile male subjects and 43 age-matched healthy volunteers with no history of systemic illness. Serum samples obtained from peripheral venous blood were analysed for TAS and TOS using Erel's automated colorimetric methods, for T-SH using the Ellman method modified by Erel and Neşelioğlu, and for PCL by enzyme-linked immunosorbent assay. Because the distributions deviated from normality, groups were compared with the Mann-Whitney U test and results are reported as median (Q1–Q3), with $p < 0.05$ accepted as significant.

Serum TOS and PCL were significantly higher in the infertile group than in controls (TOS: median 2.235 [2.185–2.265] vs 1.080 [1.050–1.110]; PCL: 4.255 [4.220–4.320] vs 2.060 [2.040–2.110]), whereas T-SH was significantly lower (6.260 [6.228–6.322] vs 12.280 [12.250–12.400]). TAS also differed significantly between the groups (5.080 [5.035–5.120] vs 10.250 [10.168–10.310]). All four comparisons were significant (Mann-Whitney U, $p < 0.001$ for each; rank-biserial $r = 1.00$), indicating an increased systemic oxidative burden and reduced antioxidant defence capacity in male infertility.

Male infertility was associated with increased systemic oxidative stress and diminished antioxidant capacity. Although the concurrent assessment of TOS, TAS, PCL and T-SH may add information on the systemic redox landscape in subfertile men, the clinical utility of these markers remains to be established. Larger, prospective, multicentre studies incorporating semen parameters are required before routine clinical use can be recommended.

Keywords: Male infertility, oxidative stress, total oxidant status, total antioxidant status, protein carbonyl, thiols, antioxidant defense, biomarkers, reactive oxygen species

Introduction

Male infertility represents a major global health concern, impacting nearly 10–15% of couples during their reproductive years, with male-related factors contributing to roughly 50% of all infertility instances (1). Although conventional semen analysis remains the cornerstone for diagnostic evaluation, its frequent failure to provide a stable and dependable correlation between sperm parameters and actual conception rates highlights the need for a deeper exploration of the biological drivers behind the condition (2). As a

result, there has been a growing emphasis in recent literature on identifying biochemical and molecular indicators that can more accurately clarify the underlying pathophysiology of infertility.

Oxidative stress is established as a key factor contributing to the etiology of male reproductive failure. Although physiological concentrations of reactive oxygen species (ROS) are essential for vital reproductive functions - including the acrosome reaction, sperm capacitation, and hyperactivation - an overproduction of these species can exert detrimental effects on spermatozoa (3,4). Elevated ROS levels are

*Corresponding Author: Veli Duman, Department of Urology, Van Yüzüncü Yıl University Faculty of Medicine
Van, Turkey

E-mail: drveli36@gmail.com

ORCID ID: Recep Eryılmaz: 0000-0002-4506-8784, Muhammed Fatih Keleş: 0000-0001-9996-1693, Rahmi Aslan: 0000-0002-4563-0386, Veli Duman: 0009-0005-1671-4889, Murat Demir: 0000-0001-5029-8800, Kasım Ertaş: 0000-0003-4300-1399, Kerem Taken: 0000-0002-4370-4222

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capable of inducing diverse forms of cellular impairment, including protein oxidation, sperm DNA instability, and lipid peroxidation within the sperm membrane. These pathological changes ultimately culminate in diminished sperm motility, abnormal morphology, and a reduced capacity for fertilization (4,10).

The occurrence of oxidative stress is rooted in a systemic disequilibrium between the production of pro-oxidant molecules and the efficacy of antioxidant defense networks. To evaluate this equilibrium, the determination of Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) has become a frequent clinical approach. Specifically, TOS serves as an indicator of the overall oxidative burden within the biological system, whereas TAS represents the collective capacity of the body's entire antioxidant repertoire (5,6). Existing literature suggests that a heightened oxidative load, coupled with a diminished antioxidant potential, is closely linked to impaired sperm function in the infertile male population (7).

Protein carbonyl levels (PCL) serve as a fundamental indicator of protein oxidation and are considered among the most dependable biomarkers for demonstrating the permanent damage inflicted by oxidative stress on cellular proteins (8). Research indicates that elevated protein carbonylation can impair fertility potential by inducing both structural and functional modifications within spermatozoa. Furthermore, total sulfhydryl (T-SH) groups represent a vital element of the antioxidant defense network, contributing significantly to the maintenance of cellular redox equilibrium through the action of protein thiols and glutathione (9).

While a broad range of research has explored the impact of oxidative stress on male reproductive health, clinical investigations simultaneously assessing systemic redox indicators - specifically TAS, TOS, PCL, and T-SH - remain relatively scarce. Consequently, this study aimed to compare these biochemical markers between infertile men and healthy controls and to clarify the role of oxidative damage in the pathophysiology of infertility.

Materials and Methods

This controlled clinical study included 40 men with infertility and 43 age-matched healthy male volunteers. Infertility was defined as the failure to achieve a clinical pregnancy after at least 12 months of regular, unprotected sexual intercourse. The duration of infertility ranged from 1 to 10 years. The major exclusion criteria were diabetes mellitus, systemic disease, renal or hepatic dysfunction,

malignancy, and chronic medication use. After written informed consent was obtained, a 5 mL peripheral venous blood sample was collected from each participant in addition to samples obtained for routine examinations. The blood samples were centrifuged at $3500 \times g$ for 10 minutes to separate the serum.

Biochemical Analyses: Serum total antioxidant status (TAS) and total oxidant status (TOS) were determined using the automated colorimetric methods described by Erel (5,6). TAS was measured at 660 nm on the basis of the decolourisation of the ABTS radical cation and expressed as mmol Trolox equivalent/L; TOS was measured at 530 nm through the oxidation of the ferrous ion-o-dianisidine complex to ferric ion in the presence of oxidants and expressed as $\mu\text{mol H}_2\text{O}_2$ equivalent/L. Total sulfhydryl (T-SH) groups were quantified by the Ellman method as modified by Erel and Neşelioğlu, based on the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid), read at 412 nm and expressed as $\mu\text{mol/L}$. Serum protein carbonyl levels (PCL) were measured by enzyme-linked immunosorbent assay at 450 nm and expressed as nmol/mg protein. All measurements were performed in duplicate on the same analytical platform, and samples from both groups were analysed within the same assay runs to eliminate between-run variability.

This study was approved by the Clinical Research Ethics Committee of Van Yüzüncü Yıl University (Decision No. 07, Date: 02/18/2026) and was conducted in accordance with the principles of the Helsinki Declaration.

Statistical Analysis: Continuous variables are expressed as median (interquartile range, Q1–Q3) and, for comparability with earlier literature, also as mean $\pm$ standard deviation. The normality of the distribution was assessed using the Shapiro–Wilk test and the homogeneity of variances using the Levene test. Normality was rejected for at least one group for all four biomarkers (Shapiro–Wilk $p=0.006$ – 0.049) and the variances were heterogeneous for TAS (Levene $p=0.008$) and PCL ($p=0.024$). Non-parametric methods were therefore used: between-group comparisons were performed with the Mann–Whitney U test, the location shift is reported as the Hodges–Lehmann median difference with its 95% confidence interval, and the effect size is expressed as the rank-biserial correlation coefficient. A p -value of <0.05 was considered statistically significant, and analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA).

Figure 1. Total Antioxidant Status (TAS)

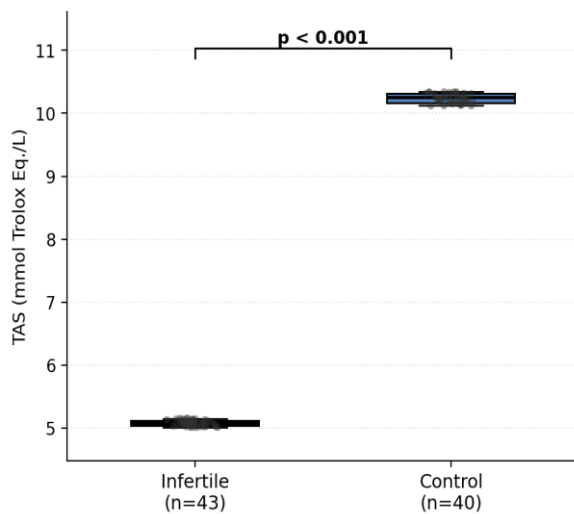


Fig. 1. Serum total antioxidant status (TAS) in the infertile and control groups

Figure 2. Total Oxidant Status (TOS)

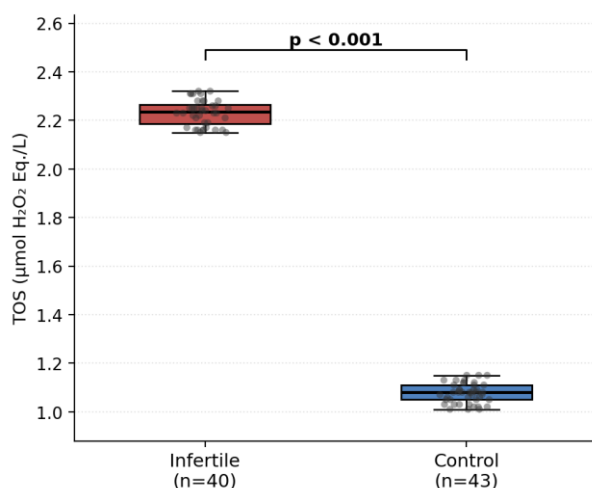


Fig. 2. Serum total oxidant status (TOS) in the infertile and control groups

Results

Clinical and semen characteristics of the infertile group are presented in Table 1. Descriptive statistics and between-group comparisons of the redox biomarkers are given in Table 2 and illustrated in Figures 1–4. Serum TOS was significantly higher in the infertile group than in controls (median 2.235 [Q1–Q3 2.185–2.265] vs 1.080 [1.050–1.110]; Hodges–Lehmann difference +1.160, 95% CI 1.130 to 1.180; $p < 0.001$), as was PCL (4.255 [4.220–4.320] vs 2.060 [2.040–2.110]; difference +2.190, 95% CI 2.170 to 2.220; $p < 0.001$). Conversely, T-SH was significantly lower in the infertile group (6.260 [6.228–6.322] vs 12.280 [12.250–12.400]; difference –6.050, 95% CI –6.090 to –6.010; $p < 0.001$), as was TAS (5.080 [5.035–5.120] vs 10.250 [10.168–10.310];

Figure 3. Total Sulfhydryl (T-SH)

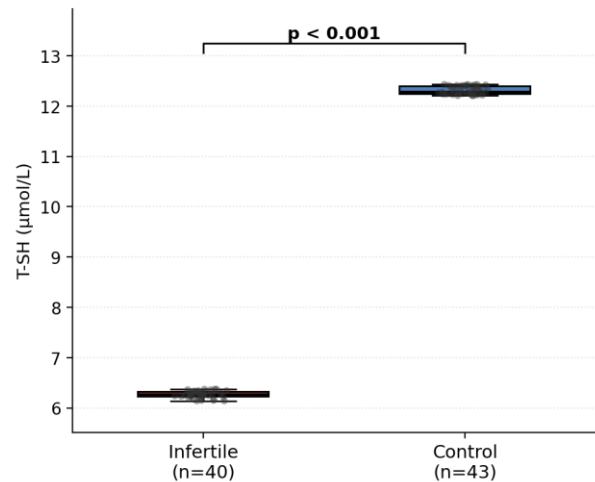


Fig. 3. Serum total sulfhydryl (T-SH) levels in the infertile and control groups

Figure 4. Protein Carbonyl Levels (PCL)

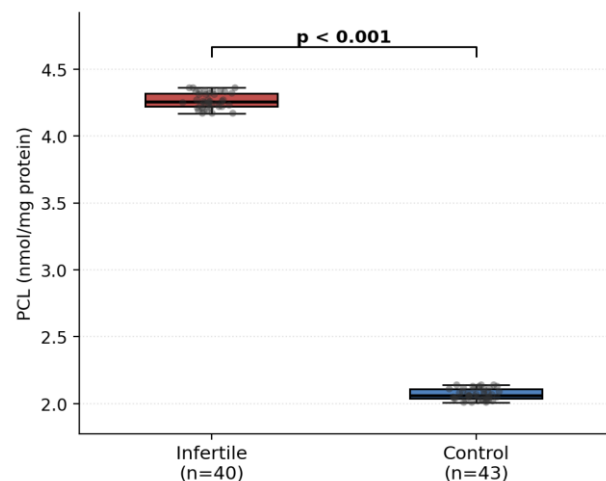


Fig. 4. Serum protein carbonyl levels (PCL) in the infertile and control groups

difference –5.150, 95% CI –5.190 to –5.120; $p < 0.001$).

Discussion

The present study focused on evaluating systemic oxidative stress by comparing the levels of TOS, TAS, PCL, and T-SH between an infertile male cohort and a healthy control population. Our findings revealed a substantial elevation in oxidative burden alongside a marked depletion in antioxidant defense capacity within the infertile group. Specifically, the observation of heightened TOS and PCL levels, coupled with significantly reduced TAS and T-SH concentrations in infertile subjects, reinforces the hypothesis that oxidative disequilibrium is a fundamental component

Table 1: Clinical and Semen Characteristics of The Infertile Group (n=40)

Characteristic	Value
Age (years), mean $\pm$ SD	28.9 $\pm$ 5.9
Age, range (years)	17–45
Current smokers, n (%)	20 (52.6)
Duration of infertility (years), mean $\pm$ SD	2.58 $\pm$ 2.11
Duration of infertility, range (years)	1–10
Varicocele or previous varicocelectomy, n (%)	4 (10.5)
Other urological pathology, n (%)*	3 (7.9)
Semen analysis outcome, n (%)	
Normozoospermic	9 (47.4)
Azoospermia	4 (21.1)
Asthenozoospermia	3 (15.8)
Oligozoospermia	1 (5.3)
Oligoasthenozoospermia	1 (5.3)

SD: standard deviation. Age was available for 36, duration of infertility for 32 and semen analysis for 19 of the participants; percentages are calculated over the available records. *Bilateral undescended testis, testicular torsion and inguinal hernia repair (one case each). Control participants were healthy volunteers recruited within the same age range and were free of systemic disease

Table 2: Serum Redox Biomarkers In The Infertile and Control Groups (Mann–Whitney U test)

Biomarker	Infertile (n=40) Median (Q1–Q3) Mean $\pm$ SD	Control (n=43) Median (Q1–Q3) Mean $\pm$ SD	Median difference	95% CI	U	p	r
TAS (mmol Trolox Eq./L)	5.080 (5.035–5.120) 5.079 $\pm$ 0.047	10.250 (10.168–10.310) 10.232 $\pm$ 0.073	-5.150	-5.190 to -5.120	1720	<0.001	1.00
TOS (μ mol H ₂ O ₂ Eq./L)	2.235 (2.185–2.265) 2.232 $\pm$ 0.053	1.080 (1.050–1.110) 1.076 $\pm$ 0.041	+1.160	1.130 to 1.180	1720	<0.001	1.00
T-SH (μ mol/L)	6.260 (6.228–6.322) 6.266 $\pm$ 0.070	12.280 (12.250–12.400) 12.316 $\pm$ 0.078	-6.050	-6.090 to -6.010	0	<0.001	1.00
PCL (nmol/mg protein)	4.255 (4.220–4.320) 4.264 $\pm$ 0.058	2.060 (2.040–2.110) 2.070 $\pm$ 0.041	+2.190	2.170 to 2.220	1720	<0.001	1.00

Q1–Q3: interquartile range; CI: confidence interval. Groups were compared with the Mann–Whitney U test; median differences are Hodges–Lehmann estimates with 95% confidence intervals. Non-parametric testing was used because the Shapiro–Wilk test rejected normality for all four biomarkers ($p=0.006–0.049$) and Levene's test indicated heterogeneous variances for TAS and PCL. r: rank-biserial correlation coefficient

in the pathophysiological development of male infertility.

Oxidative damage is widely acknowledged as a fundamental pathway in the clinical progression of male reproductive failure. Although the maintenance of physiological concentrations of reactive oxygen species (ROS) is vital for normal sperm activity, a surplus of ROS triggers detrimental cascades such as DNA fragmentation, protein carbonylation, and lipid peroxidation within the spermatozoa (3,10). These

pathological alterations ultimately impair sperm kinetics and diminish overall reproductive potential.

The observation of heightened TOS concentrations in the infertile cohort of our study points toward an intensified systemic oxidative burden. This outcome aligns with previous findings documented in the scientific literature. Specifically, Agarwal et al. demonstrated that elevated indicators of oxidative stress in subfertile males were linked to sperm DNA fragmentation (7). In a similar vein, Durairajanayagam highlighted that an escalation in oxidative stress levels

among infertile individuals leads to a deterioration in sperm functionality (11).

The observed reduction in TAS concentrations, which reflects the body's antioxidant shielding efficiency, implies a failure of the defense mechanisms to adequately counteract the heightened oxidative burden. This state of impaired antioxidant resilience in subfertile males is corroborated by the research of Agarwal et al. (7). On the other hand, Majzoub and Agarwal have indicated that the administration of antioxidant-based treatments might enhance seminal quality and parameters within certain patient subgroups (12).

Furthermore, the diminished T-SH concentrations observed in our research signify a compromised antioxidant buffering capacity. Total sulfhydryl groups are essential mediators in preserving cellular redox stability. Our findings align with prior investigations suggesting that a reduction in T-SH availability is a characteristic feature of subfertile clinical profiles, reflecting an exhausted defense system against oxidative insults (9).

The finding of reduced total sulfhydryl concentrations reflects a compromised thiol-based buffering system, which is a fundamental element of the body's antioxidant machinery. A decline in these thiol groups renders cellular structures more vulnerable to oxidative injury (9). Previous evidence in the scientific literature has established a clear correlation between diminished thiol availability and the occurrence of male infertility (13).

Beyond cellular markers, oxidative stress significantly compromises the structural integrity of sperm DNA. Elevated ROS generation triggers DNA fragmentation, which subsequently undermines the probability of successful fertilization. Evidence provided by Simon et al. has established a robust correlation between the extent of genomic damage in spermatozoa and reproductive failure (15). Moreover, such molecular instability can adversely interfere with the clinical efficacy and outcomes of assisted reproductive techniques (16).

Therapeutic strategies incorporating antioxidants are frequently employed to mitigate the impact of oxidative stress in the subfertile male population. Specifically, the administration of agents including L-carnitine, coenzyme Q10, and vitamins C and E has been documented to enhance seminal parameters (17). Nevertheless, current clinical perspectives highlight that therapeutic efficacy varies significantly among individuals, necessitating a personalized approach to antioxidant supplementation rather than a standardized protocol.

Indicators of oxidative stress possess substantial clinical utility for the diagnostic assessment of male reproductive failure. Integrating these biochemical markers alongside traditional semen analysis can provide deeper insights into the metabolic foundations of infertility. Specifically, the concurrent evaluation of TAS, TOS, T-SH, and PCL concentrations offers a more holistic and multifaceted perspective on the systemic redox equilibrium (18).

The present study has several limitations. First, the sample size was modest and the design was single-centre and cross-sectional, so causality cannot be inferred and the findings require confirmation in independent cohorts. Second, the study was retrospective with respect to clinical data: semen analysis results were available for a subset of participants and were recorded as diagnostic categories rather than as quantitative WHO parameters, which precluded correlation analyses between individual semen variables and redox markers and prevented separate analysis of infertility subtypes. Third, lifestyle and anthropometric variables other than smoking were not available, so residual confounding cannot be excluded. Fourth, all measurements were performed in a single laboratory; external validation of the assay results in an independent setting would strengthen confidence in the magnitude of the differences observed. Nevertheless, the controlled design and the simultaneous assessment of four complementary redox markers in the same individuals represent important methodological strengths.

In conclusion, this study demonstrates that male infertility is associated with a significant increase in systemic oxidative burden, reflected by higher TOS and PCL, together with a reduction in antioxidant defence, reflected by lower TAS and T-SH. The concurrent measurement of these four markers characterises the systemic redox imbalance in subfertile men more completely than any single marker alone. However, these findings derive from a single-centre study of modest size in which quantitative semen parameters were not available for correlation analysis, and the clinical utility of these biomarkers therefore remains to be established. Larger prospective multicentre studies integrating redox markers with quantitative semen analysis and reproductive outcomes are required before they can be recommended for routine clinical use.

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