

## Is oxidative stress an etiologic factor in idiopathic male infertility?

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**Aim:** The total antioxidant capacity (TAC) and total peroxide (TP) levels in the blood and seminal plasma of idiopathic infertile patients were compared with those of fertile controls to determine the role of oxidative stress in the etiology of idiopathic male infertility.

**Material and methods:** Thirty-two idiopathic infertile males and 30 healthy, married, age-matched control subjects were included in the study. TAC and TP levels were measured in the blood and seminal plasma, and sperm parameters were studied in both groups.

**Results:** Sperm parameters were significantly impaired in the study group compared with the controls. Blood plasma TAC and TP levels in the patient group were  $1.46 \pm 0.62$  mmol Trolox equiv./L and  $8.38 \pm 0.87$  mmol H<sub>2</sub>O<sub>2</sub>/L, respectively, compared with  $1.72 \pm 1.70$  mmol Trolox equiv./L and  $8.59 \pm 1.08$  mmol H<sub>2</sub>O<sub>2</sub>/L in the control group. Seminal plasma TAC and TP levels in the patient and control groups were  $13.48 \pm 2.03$  mmol Trolox equiv./L,  $7.79 \pm 0.38$  mmol H<sub>2</sub>O<sub>2</sub>/L,  $14.06 \pm 2.30$  mmol Trolox equiv./L, and  $7.84 \pm 0.51$  mmol H<sub>2</sub>O<sub>2</sub>/L, respectively. TAC and TP levels in the blood and seminal plasma did not differ significantly between the patient group and the control group.

**Conclusion:** These findings suggest that oxidative stress is not the cause of impaired sperm parameters in idiopathic infertile men.

**Key words:** Idiopathic infertility, total antioxidant capacity, total peroxide, sperm parameters

### Oksidatif stres idyopatik erkek infertilitesinde etyolojik bir faktör müdür?

**Amaç:** Bu çalışmada, idyopatik erkek infertilite etyolojisinde oksidatif stresin rolünü araştırmak için idyopatik infertil hastaların kan ve semen plazmasındaki toplam antioksidan kapasite (TAC) ve toplam peroksit (TP) düzeyleri fertil kontrollerle karşılaştırıldı.

**Yöntem ve gereç:** 32 idyopatik infertil erkek ve 30 sağlıklı, evli ve benzer yaş grubunda kontrol bireyi çalışmaya alındı. Hasta ve kontrollerde kan ve seminal plazma TAC ve TP düzeyleri ölçüldü, sperm parametreleri incelendi.

**Bulgular:** Hasta grubunda sperm sayı, hareket ve morfoloji ölçümleri kontrol grubundan anlamlı şekilde düşük bulundu. Kan plazmasında TAC ve TP düzeyleri, hasta grubunda  $1,46 \pm 0,62$  mmol Trolox equiv./L ve  $8,38 \pm 0,87$  mmol H<sub>2</sub>O<sub>2</sub>/L iken kontrol grubunda  $1,72 \pm 1,70$  mmol Trolox equiv./L ve  $8,59 \pm 1,08$  mmol H<sub>2</sub>O<sub>2</sub>/L olarak bulundu. Semen plazmasında TAC ve TP düzeyleri, hasta grubunda  $13,48 \pm 2,03$  mmol Trolox equiv./L ve  $7,79 \pm 0,38$  mmol H<sub>2</sub>O<sub>2</sub>/L, kontrol grubunda ise  $14,06 \pm 2,30$  mmol Trolox equiv./L ve  $7,84 \pm 0,51$  mmol H<sub>2</sub>O<sub>2</sub>/L idi. Hem semen hem de kan plazmasında oksidatif/antioksidatif parametreler açısından hasta ve kontroller arasında anlamlı fark belirlenmedi.

**Sonuç:** Bu çalışmanın sonuçlarına göre, idyopatik infertil hasta grubunda, spermiyogram parametrelerindeki düşüklüğün oksidatif stres ile açıklanamayacağı ve altta yatan başka faktörlerin aranması gerektiği kanaatine varıldı.

**Anahtar sözcükler:** İdyopatik infertilite, total antioksidan kapasite, total peroksit, sperm parametreleri

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## Introduction

Approximately 15% of all couples trying to conceive are clinically infertile, and male-factor infertility is involved in half of those cases (1). While several situations can cause infertility, such as varicocele, obstructive, hormonal, and immunologic pathologies, identifiable causes cannot be found in over 25% of infertile males, and these cases are referred to as idiopathic infertility (1,2).

Although considerable progress has been made toward understanding sperm physiology and the biology of gamete interaction, still more work in cellular and molecular levels is needed to exhibit pathology and to develop the diagnostic and treatment methods for the clinical setting, particularly in idiopathic infertile cases (3).

Electrons in the outer orbital of an atom or molecule are generally available to form shared molecular bonds with other atoms or molecules, and thereby create a stable structure. This stable structure is disturbed when an atom or molecule takes one electron from outside or when they gain one electron, or by homolytic disintegration. Structures that have unpaired valence shell electrons are called reactive oxygen species (ROS). ROS are produced in normal conditions within organism, but are immediately eliminated in endogen and exogen antioxidant mechanisms, and in some situations act to the benefit of the organism (4,5). Antioxidants and oxidants interact with each other in the blood plasma. This combined interaction produces a much stronger effect than the sum of the individual effects. Many antioxidant and oxidant molecules cannot be individually measured, and such measurement is very expensive; so total antioxidant capacity (TAC) and total peroxide (TP) are more useful for evaluation of oxidative status (6,7).

The sperm membrane plays an important role in fusion and the acrosome reaction, which is essential for fertilization. Because of this feature, there is an important correlation between pathologies of the sperm membrane and infertility. The presence of abundant unsaturated fatty acids in the membrane of human sperm cells and the high affinity of ROS to these structures are important in maintaining the oxidant and antioxidant balance during normal

functions of the sperm (4,5,8). An imbalance in favor of oxidation causes structural anomalies in sperm cells and a reduction in the conjugation capacity of sperm and oocytes, which would cause a reduction in the fertilization rate (9,10).

The causes of infertility in a significant proportion of men are unknown, and therefore these situations are temporarily referred to as idiopathic infertility. Recent research has focused on the mechanisms of this side of infertility (4,5). In this study, oxidative status in idiopathic infertile patients was compared with that in fertile controls to determine whether oxidative stress is an etiological factor in idiopathic male infertility.

## Materials and methods

The study was approved by the Institutional Review Board of the School of Medicine, Harran University. All subjects were informed about the study protocol and written consent was obtained.

### Subjects

This prospective study included 135 consecutive male patients attending the andrology and infertility clinic at our medical faculty for infertility evaluation. All patients were evaluated with a complete medical history and physical examination that focused on the secondary sex characteristics and congenital and/or acquired genital malformations. Men were excluded if they had been infertile for less than 1 year. Semen samples were analyzed as described below. Each patient's hormone profile was studied, including FSH, LH, testosterone, and prolactin. In cases where additional malformations were suspected and the physical examination was insufficient or suspicious, scrotal, transrectal, and/or abdominal ultrasonography was performed. Infertility cases related to known causes, such as varicocele, leukocytospermia, hormonal, and/or obstructive pathologies were excluded from the study. The remaining patients were regarded as idiopathically infertile. Thirty age-matched healthy males who had fathered a child in the previous year and had normal semen parameters according to World Health Organization (WHO) criteria (11) were enrolled as a control group.

**Eligibility Criteria:**

- i. aged above 20 or below 45 years
- ii. no clinic varicocele
- iii. no leukocytospermia in semen analysis
- iv. no hormonal disorder
- v. no urogenital infection
- vi. non-smoker
- vii. no intake of multivitamin drugs in the previous 2 weeks

**Collecting and Analyzing Samples****Semen analysis**

Two semen samples were taken from each patient at an interval of between 1 and 3 weeks. When a 20% difference at the sperm concentration, motility, and/or morphology was found between the 2 samples, a third sample was taken. Semen samples were produced by masturbation and collected into clean glass containers after a period of 48-72 h of sexual abstinence. Seminograms including the volume of ejaculate (mL), sperm concentration ( $\times 10^6/\text{mL}$ ), forward motility (%), and morphology (% of atypical forms) after semen liquefaction were performed according to WHO guidelines (11). Samples were stored at 37 °C and analyzed within 2 h, by the same biologist. Sperm morphology was evaluated with Giemsa staining, and number and movement were evaluated with a Makler camera. Sperm motility was classified into 4 classes according to WHO guidelines: "a" rapid progressive motility, "b" slow progressive motility, "c" non-progressive motility, and "d" no motility.

Aliquots of liquefied semen were centrifuged at 4000 rpm for 20 min; their supernatant parts were stored at -80 °C to study their total antioxidant capacity and total peroxide levels.

**Blood samples and analysis**

The participants, who had fasted for 10-12 h, were placed in a supine position and blood samples of approximately 5 cc were withdrawn from a cubital vein into heparinized tubes. All blood samples were taken in the morning, between 0800 and 1000 hours. Blood samples were immediately centrifuged at 3000 rpm, for 10 min. The plasma was separated and stored

at -80 °C until analysis. TAC and TP levels were measured at the same time for all samples.

**Measurement of Total antioxidant capacity**

Total antioxidant capacity levels of stored blood plasma and seminal plasma samples were measured with a total antioxidant capacity kit and with an autoanalyzer (Abbott Aeoroset, USA) (7). Trolox was used as the assay standard and the results were expressed as mmol Trolox equiv./L.

**Measurement of Total peroxide**

Total peroxide levels of blood and semen plasma samples were determined using the 'FOX2' method with minor modifications (6) and the results were expressed as mmol  $\text{H}_2\text{O}_2/\text{L}$ .

**Statistical analysis**

Statistical analysis of the data was carried out with SPSS (Statistical Package for the Social Sciences, version 11.5, for Windows, SPSS, Inc). Student's t-tests were used to compare differences between the groups because the variables were normally distributed. Student's t-test for independent samples was used to compare differences between the patient and control groups according to mean levels of TAC and TP. Student's t-test for paired samples was used to compare mean levels of TAC and TP in the seminal and blood plasma samples in any group. A 95% confidence interval was used. P values less than 0.05 were considered statistically significant.

**Results**

The results are given as mean  $\pm$  standard deviation of mean. One hundred three patients out of 135 consecutive infertile patients were excluded from the study. The reasons for exclusion of these 103 patients are summarized in Table 1. The study included 32 patients diagnosed according to the criteria for idiopathic infertility. The mean age of the idiopathic infertile patient group and the control group was  $31.03 \pm 4.94$  (range 21-39) and  $31.30 \pm 3.95$  (range 22-40) years, respectively. The differences between the groups were not statistically significant.

The semen characteristics of the idiopathic infertile patient and control groups are shown in Table 2. Sperm concentration ( $\times 10^6/\text{mL}$ ), percentage of

Table 1. Exclusion reasons of 103 patients from the study.

Number of patients (%)	Exclusion reason
47 (45.63)	Varicocele
17 (16.50)	Leukocytospermia
6 (5.83)	Hormonal reasons
3 (2.91)	Obstructive reasons
30 (29.13)	Other (age, female factor, urinary infection, etc.)

motility (a + b), percentage of normal forms, and volume (cc) in the patient group were significantly lower than in the control group.

Mean TAC and TP values of seminal and blood plasma samples are shown in Table 3 for the study group and the controls. In both groups, TAC was significantly higher (approximately 8- to 9-fold) in seminal plasma compared to blood plasma and TP levels were lower in seminal plasma compared to

blood plasma. The differences between seminal plasma and blood plasma according to mean TAC and TP values were statistically significant in both groups. However, the mean TAC and TP values for both seminal and blood plasma samples were not significantly different between the patient and control groups.

## Discussion

In the past, particularly in Eastern societies, women were blamed for infertility. However, current understanding of the causes of infertility recognizes that male infertility is a factor in 50% of cases. No cause can be diagnosed in approximately 25% of infertile males, which is termed 'idiopathic infertility' (1). In the present study group, no cause of infertility could be identified in 32 (23.7%) of 135 infertile patients and these cases were regarded as 'idiopathic infertility'.

Table 2. Semen characteristics of idiopathic infertile patient and control groups.

	Patient	Control	P
Concentration ( $\times 10^6$ /mL)	24.59 $\pm$ 10.0	51.26 $\pm$ 11.86	<0.001
Motility (a + b) (%)	35.53 $\pm$ 18.76	56.06 $\pm$ 7.25	<0.001
Morphology (%)	44.75 $\pm$ 18.15	57.16 $\pm$ 9.65	0.001
Volume (cc)	2.83 $\pm$ 0.51	3.80 $\pm$ 0.77	<0.001
pH	7.43 $\pm$ 0.35	7.35 $\pm$ 0.32	0.334

P; Student's t-test for independent samples

Table 3. TAC and TP values of semen and blood plasma in idiopathic infertile patient and control groups.

Parameter		Seminal plasma	Blood plasma	P <sub>1</sub>
Total Antioxidant Capacity (mmol Trolox equiv./L)	Patients	13.48 $\pm$ 2.03	1.46 $\pm$ 0.62	<0.001
	Controls	14.06 $\pm$ 2.30	1.72 $\pm$ 1.70	<0.001
	P <sub>2</sub>	0.303	0.505	
Total Peroxide (mmol H <sub>2</sub> O <sub>2</sub> /L)	Patients	7.79 $\pm$ 0.38	8.38 $\pm$ 0.87	0.001
	Controls	7.84 $\pm$ 0.51	8.59 $\pm$ 1.08	0.003
	P <sub>2</sub>	0.680	0.411	

P<sub>1</sub>, Student's t-test for independent samples

P<sub>2</sub>, Student's t-test for paired samples

In order to determine pathophysiology and to increase the success of treatment, particularly in idiopathic infertile cases, studies are performed at cellular and molecular levels (3,5,12-14). One of the research areas is the relationships between anomalous sperm parameters and ROS (4,5,15,16). Oxidative stress may play a role in a number of conditions known to be detrimental to male fertility. These conditions include exposure to environmental and industrial toxins (17), gonadotoxic chemotherapy (18), ionizing radiation (19), aging (20), varicocele (21,22), testicular torsion, infection, and inflammation (23,24). The oxidative stress environment that is created by ROS in the seminal plasma can be toxic for sperm. The presence of unsaturated fatty acids in the sperm cell membrane, the affinity of ROS to these structures, lipid peroxidation following the interaction of sperm membrane lipid layer and oxy radicals, and potential anomalies of the membrane lipid structure are thought to be factors in the relationship between ROS and infertility (4,5,15,16).

Idiopathic infertility in males has been linked to oxidative stress by several researchers. The primary reason for this connection is the observation of morphologically abnormal sperm, commonly identified in the idiopathic infertile male population, and a reduced antioxidant capacity (10,25,26). Even men with normozoospermic idiopathic infertility exhibit significantly higher seminal ROS production and lower antioxidant capacity than fertile men (14,27), for reasons not yet understood (5).

In contrast, some previous studies suggest that there is no relationship between ROS and infertility, or that ROS does not differ between infertile patients and controls (26,28,29). Yaman et al. (28) did not find any statistical difference for xanthine oxidase and malondialdehyde concentrations in testicular tissue between a control group who had normal spermatogenesis and infertile patients who had Sertoli cell-only syndrome. They also proposed that there must be other explanatory factors, beside ROS. Hsieh

et al. (29) did not find any significant difference between fertile and infertile patients in relation to the activities of seminal plasma superoxide dismutase (SOD) and they suggested that there is no relationship between seminal plasma antioxidant status and sperm number and movement.

In our study, TP and TAC measurements did not show any significant difference between infertile patient and control groups, whereas a difference was observed in sperm parameters. The association of sperm motility defect and seminal oxidative stress is not clear, but abnormal morphology is highly associated with the production of ROS in idiopathic infertile men in the literature (16,30). The lack of oxidative stress in our idiopathic infertile patient group may be related to sperm parameters in the normal range according to the latest WHO criteria, except for motility. These findings in the idiopathic infertile patient group suggest that low sperm parameters cannot be explained by oxidative stress and there must be additional causal factors.

There is little information in the literature comparing seminal plasma and blood plasma for TAC and/or TP levels. Fingerova et al. (31) reported that the level of TAC in seminal plasma was nearly 1.5 times higher than that in blood plasma. In the present study, the seminal plasma TAC levels were approximately 8-9 times higher than in blood plasma in both patient and control groups. When the same comparison was made for TP levels, it was found to be significantly low in semen plasma. This high antioxidant capacity in seminal plasma seems to be sufficient to prevent increased ROS and also to prevent secondary (to increased ROS) spermatozoa damage.

Consequently, the results of the present study suggest that anomalies of sperm parameters in idiopathic infertility cannot be explained by oxidative stress. The literature as a whole provides contradictory findings and further research with larger samples will help us to gain a better insight into this problem.

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