AMERICAN JOURNAL OF BIOSCIENCE AND CLINICAL INTEGRITY
https://biojournals.us/index.php/AJBCI
ISSN: 2997-7347

Article

Seminal plasma Oxidative stress (MDA) and Total antioxidant capacity (TAC) its clinical relation to testosterone and prolactin hormon in oligozoospermia men

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Citation: Salih A. T. Mohammad Alwafi H. A.-K., Khosho E. Z. Seminal plasma Oxidative stress (MDA) and Total antioxidant capacity (TAC) its clinical relation to testosterone and prolactin hormon in oligozoospermia men. American Journal Of Bioscience And Clinical Integrity 2026, 3(2), 27-39

Received: 10th Nov 2025
Revised: 21th Dec 2025
Accepted: 14th Jan 2026
Published: 06th Feb 2026

Abstract: This case-control study aimed to investigate the relationship between reproductive hormones, oxidative stress markers, and antioxidant capacity in men with oligozoospermia. A total of 200 participants were enrolled, comprising 100 oligozoospermic men and 100 fertile controls. Seminal levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were assessed alongside serum concentrations of testosterone, prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol. Compared to controls, the oligozoospermic group exhibited significantly higher oxidative stress, as indicated by elevated MDA levels ($p<0.001$), and significantly lower antioxidant capacity, reflected by reduced TAC levels ($p<0.001$). Hormonal analysis revealed significantly increased testosterone ($p<0.001$) and decreased prolactin ($p<0.001$) levels in infertile men. Strong inverse correlations were observed between MDA and testosterone, as well as between TAC and prolactin. These findings suggest that oligozoospermia is associated with a distinct biochemical profile characterized by heightened oxidative damage, impaired antioxidant defense, and altered endocrine regulation. The study highlights the potential clinical utility of integrating oxidative and hormonal biomarkers for a more comprehensive diagnostic approach to male infertility, reflecting its multifactorial pathophysiology.

Keywords: Oligozoospermia, Oxidative Stress, Malondialdehyde, Testosterone, Male Infertility

Introduction

Male infertility affects 10–15% of couples worldwide, with a male factor involved in approximately half of cases. The World Health Organization (WHO) criteria define oligozoospermia as a sperm concentration below 15 million/mL. Oxidative stress contributes to male infertility, damaging sperm membrane proteins and lipids and leading to decreased motility and DNA fragmentation. Seminal plasma antioxidants, including superoxide dismutase (SOD) and catalase, protect sperm from oxidative damage [1].

However, some studies report increased SOD activity in infertile men, especially in oligozoospermic individuals. Other factors, such as the total antioxidant capacity of the semen, also influence sperm quality, and lower total antioxidant capacity has been associated with oligozoospermia and asthenozoospermia [2]. Testosterone and prolactin are crucial for male fertility and influence semen parameters. Testosterone stimulates testicular growth, Leydig cell differentiation, and spermatogenesis, while prolactin enhances spermatogonial growth and testosterone production. Prolactin deficiency in males impairs sperm production and contributes to oligozoospermia [1].

Both hormones can modify lipid peroxidation and the oxidative status of various tissues. Testicular tissue from hyperprolactinaemic rats has exhibited increased malondialdehyde (MDA) levels, reflecting higher peroxidation, and decreased antioxidant enzymatic activities [3, 4]. These observations suggest that oxidative stress may affect the hormonal milieu and that the trace elements involved in antioxidant protection and the pathogenesis of oxidative stress are essential for fertility [5, 6]. In light of these interrelationships, this study investigated the association between seminal plasma markers of oxidative stress MDA and the total antioxidant capacity (TAC) and hormonal levels of testosterone and prolactin in oligozoospermic men and matched controls. Oligozoospermic participants were hypothesized to have higher MDA and lower TAC compared to controls. Moreover, lower testosterone and prolactin concentrations with decreased sperm counts were expected to correlate with higher MDA and lower TAC in oligozoospermic subjects [7, 8].

Background on Male Infertility

Infertility affects 15 % of couples, with male factor involved in 50 % of cases and exclusively implicated in 20 % [9]. Oligozoospermia is one of the most noted causes of male infertility; according to the WHO, it is diagnosed when the sperm concentration is <15 million spermatozoa per milliliter of semen. Seminal plasma, the fluid portion of semen, consists of secretions from the prostate gland, seminal vesicles, and bulbourethral glands. These secretions provide nutrients and transport medium for the spermatozoa as well as various biomolecules that help to regulate the physiological condition of the spermatozoa [10]. Infertile men show increased seminal plasma markers of oxidative stress and diminished antioxidant capacity, with higher concentrations of malondialdehyde (MDA) and lower total antioxidant capacity (TAC) relative to fertile controls. Increased oxidative stress may therefore be associated with the etiology of male infertility. Oxidative stress may also alter the hormonal profile in the infertile male, triggering physiological alterations in the reproductive organs and affecting the quality of gametes.

Fertility is under the control of a myriad of hormones, testosterone and prolactin being among the most important. Testosterone exhibits a wide range of effects on the male reproductive system, including modulation of libido, sperm production, and the formation of male secondary sex characteristics, such as the growth of the beard and deepening of voice [11]. In particular, testosterone influences the quality of the spermatozoa and embryological development thereafter. Prolactin, with a relative abundance nearly equal to that of testosterone, acts through a receptor which is situated near the Leydig cells of the male reproductive organ and possesses similar physiological effects to testosterone. Elevated production of prolactin is commonly found in oligozoospermic patients [12].

Role of Oxidative Stress in Oligozoospermia

The dilemma of male infertility is becoming critical worldwide, primarily due to its progressive augmentation in contemporary society. As per the WHO report, male factors, alone or in combination with female factors, contribute to around half of the total infertility cases. Oligozoospermia, a male factor responsible for infertility, is defined as a semen volume less than 1.5 mL and sperm concentration lower than 15 million per mL. As per credible estimates, 30% of male infertility is solely attributable to oligozoospermia [10]. Adequate sperm concentration is paramount for normal male fertility, with limitations beyond 250 M/mL rarely resulting in normal mating, making the endocrine regulation of spermatogenesis critical. Maintenance of proper circulating concentrations of hormones, including testosterone, dihydrotestosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estradiol, thyroid-stimulating hormone, thyroid hormone, and cortisol is vital for normal male fertility. [13]

Oxidative stress results from an imbalance between oxidants and antioxidants, leading to peroxidation of lipids, alteration of protein structure and function, DNA strand breakage, loss of calcium homeostasis, reduction in ATP levels, and ultimately cell death and tissue injury [2]. Male genital accessory organs act as a barrier against oxidative stress and maintain the viability, motility, and morphology of spermatozoa. But, seminal plasma derived from the epididymis is vulnerable to external oxidative attack and ultimately leads to sperm dysfunction. Redox reactions involving lipid peroxides and production of malondialdehyde (MDA) are used to assess damage to spermatozoa due to oxidative stress. Glutathione (GSH), superoxide dismutase (SOD), and catalase are utilized to determine the antioxidant mechanism. Seminal plasma total antioxidant capacity (TAC) employed to study antioxidant systems. Given the hormonal and oxidative stress interplay, the present investigation aims to assess the association between seminal plasma MDA, TAC, and testosterone, prolactin in blood of oligozoospermic men. [14]

Hormonal Regulation and Male Fertility

Oligozoospermia in men is associated with alterations in testicular function, as reflected in changes in semen parameters and hormone levels. In this context, male fertility relies on an optimal hormonal environment, where testosterone and prolactin are essential regulators capable of influencing sperm production and semen quality [9]. According to earlier research, the male spermatozoon and the accessory glands are subject to oxidative harm, with increasing reactive oxygen species correlating negatively with sperm vitality, motility, and morphology. Affected individuals exhibit aberrances in the hypothalamic-pituitary-gonadal axis, not infrequently together with increases in other inflammatory markers. Research studies have demonstrated the dual function of testosterone as pro- and anti-oxidant, while prolactin outcompetes other sex steroids for spermatozoon receptors. There exists a gap in the knowledge of oxidative mechanisms underlying male infertility, particularly concerning the interrelations between hormonal milieu and seminal oxidative stress markers in oligozoospermic men [15, 16].

Oxidative stress in seminal plasma is associated with compromised semen parameters and male infertility; in particular, increased malondialdehyde (MDA) levels and diminished total antioxidant capacity (TAC) have been reported in infertile males [1]. Significant changes of these parameters were detected also in the cohort, as the study group showed a mean MDA value of 4.44 pg/ml which was almost twice those measured for controls and a mean TAC level of 168.30 μmol/l that was about one half compared to controls (mean =327.80 μmol/l), with a negative correlation between MDA and total motility limiting sperm count and progressive motility, while it correlated positively with breeding potential tests and higher concentrations [17, 18].

Research Objectives of the Study

1. To compare and characterize the levels of key oxidative stress biomarkers—particularly malondialdehyde (MDA) and total antioxidant capacity (TAC)—in the seminal plasma of men with oligozoospermia against fertile controls.
2. To evaluate and contrast the serum concentrations of pivotal reproductive hormones—specifically testosterone and prolactin, alongside FSH, LH, and estradiol—between oligozoospermic and fertile male groups.
3. To assess the significant interrelationship observed between elevated seminal oxidative stress (MDA) and increased testosterone levels, as well as between reduced antioxidant capacity (TAC) and diminished prolactin levels, in men with oligozoospermia.

Literature Review

Despite technological advances in assisted reproductive techniques, male factor infertility remains a clinical challenge. Approximately 50% of cases are idiopathic, and one-third of these have low sperm counts (oligozoospermia) [1]. Oxidative stress, a phenomenon of increased concentrations of reactive oxygen species (ROS) due to a low antioxidant capacity, is implicated in male fertility and has been documented in both idiopathic and clinically defined infertility. In spermatozoa, excessive oxidative stress causes lipid peroxidation of membrane phospholipids, leading to membrane damage, decreased motility, altered fertilization capability, and DNA damage.

Furthermore, oxidative stress in the reproductive system can lead to Leydig cell damage, thereby reducing testosterone secretion [19]. Because both low testosterone levels and high oxidative stress indicators are associated with abnormal semen parameters, it is important to investigate the interactions between the two in oligozoospermic men. Prolactin, another important hormone that regulates spermatogenesis, is also related to oxidative stress. Infertile men have higher prolactin levels than fertile men, and increased prolactin secretion in oligozoospermic men positively correlates with immotility and negatively correlates with normal morphology. A better understanding of the relationship between oxidative stress and hormonal profiles, particularly testosterone and prolactin, in oligozoospermic men with normal clinical profiles may improve clinical diagnosis and therapeutic options.

Male Infertility: Prevalence and Etiology

Male infertility affects perspective parents seeking to conceive. With approximately 30% of infertility cases attributed to male factors, infertility is beyond the capacity of medical intervention. Oligozoospermia is the most common condition. Seminal plasma oxidative stress can negatively affect semen quality, and hormonal parameters are also known to differ in men with infertility or sexual dysfunction. However, studies exploring the relationship between oxidative stress markers in seminal plasma and testosterone and prolactin levels in an oligozoospermic cohort remain scarce. Infertility is a disease defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [7].

Male infertility is diagnosed when no clinical pregnancy occurs within 12 months of unprotected intercourse, at least 30% of samples having testosterone concentrations below the reference interval, or when any sample has total testosterone concentrations below 18.5 nmol/L. It is often underestimated in general populations because males typically do not seek medical assistance. Males are responsible for about 30% of infertility cases, and overall, sperm counts have decreased over the last 20 to 30 years. Oligozoospermia, defined by sperm counts below 15 million/mL and commonly associated with other parameters past the World Health Organization reference values, is the most prevalent condition [20].

Oxidative Stress Biomarkers in Seminal Plasma

Semen quality is determined in part by oxidative stress, which is due to an imbalance between pro-oxidants and antioxidants. An increase in oxidative stress in oxidative-sensitive biological fluids such as seminal plasma may, therefore, be expected to alter spermatozoa and semen quality parameters, since their production leads to the formation of reactive species that participate in lipid peroxidation of membranes and consequently affect membrane permeability, motility, maturation, sperm–zona interaction, DNA integrity, and membrane integrity [19].

Lipid peroxidation can be evaluated by measuring levels of malondialdehyde (MDA), one of the most abundant by-products formed during lipid peroxidation and the simplest and most frequently used marker for studying lipid peroxidation. A low concentration of antioxidants or an increase in oxidative agents may also induce oxidative damage in semen. The total antioxidant capacity (TAC) of seminal plasma gives an indication of the status of the seminal plasma in counteracting these deleterious agents. A low TAC level can, therefore, contribute to oxidative stress and may reflect a lack of essential nutrients, particularly micronutrients, for supporting cellular antioxidant defense and thereby contribute to male infertility [20].

Testosterone is secreted mainly by Leydig cells and is essential for spermatogenesis; its deficiency is associated with impaired spermatogenesis and increased testicular degeneration. Prolactin is also important in male fertility, but its role remains controversial [21]. Higher prolactin concentrations are thought to be harmful to sperm, whereas lower prolactin levels seem to be more highly correlated with the maturity of male germ cells, sperm abnormality, and biochemical indicators of reproductive function. In patients with oligozoospermia, a decrease in testosterone concentrations is expected, while the effect of prolactin on oxidative stress is more complex, since it may influence the activity of different antioxidants in semen and in different directions. Whether and how these factors are related to seminal oxidative stress remains to be fully elucidated [22].

Hormonal Profiles in Infertile Men

Oligozoospermic men displayed low testosterone and high prolactin levels, correlating positively with malondialdehyde and negatively with total antioxidant capacity. Male infertility is often characterized by hormonal imbalances. Oxidative stress contributes to pathophysiological conditions, and malondialdehyde (MDA) may adversely affect spermatogenesis. Furthermore, changes in prolactin and testosterone affect the seminiferous tubule microenvironment. A relationship may exist between oxidative stress and hormonal homeostasis disturbance in men with poor sperm parameters. Follicle-stimulating hormone, luteinizing hormone, and testosterone stimulate the synthesis of glycoprotein by Sertoli cells, supporting sperm maturation, while prolactin, in a direct or indirect manner, plays a complementary role [23].

Therefore, prolactin and testosterone are expected to display positive relationships with semen parameters, an expectation supported by numerous studies. The role of estradiol is more controversial, they have raised the possibility of a significant role in males. Simultaneously, high prolactin levels have been linked to spermatogenesis inhibition and sperm motility impairment. The available evidence suggests that alterations in these hormones play a marked role in the mechanisms underlying male infertility. Several studies have investigated the relationship between sex steroid hormones and total antioxidant capacity (TAC) and glutathione and catalase levels in testicular tissue; in general, these studies report positive relationships. Hormonal changes frequently observed in infertile men may also influence oxidative status in the seminal fluid. However, research focusing on the interaction between hormonal homeostasis and oxidative stress in the semen of oligozoospermic men is lacking [24].

Interplay Between Hormones and Oxidative Stress

Semen quality is intricately connected with the male endocrine milieu, particularly the hypothalamic-pituitary-gonadal (HPG) axis. Adequate testosterone levels and the secretory activity of the testes and accessory glands are critical for normal spermatogenesis, libido, and erectile response to sexual stimulation. Prolactin, primarily recognized for its role in lactation, also influences male reproductive functions and is necessary for normal fertility. Correlations between the levels of important reproductive hormones, primarily testosterone and prolactin, and the state of local oxidative stress in the seminal fluid environment are not yet fully elucidated. Current literature suggests a possible link between male infertility, particularly through parameters that influence fertility, such as oligozoospermia, and increased oxidative stress at the level of the seminal plasma [25].

Oxidative stress becomes an exacerbating factor when hormonal alterations push the local physiology toward an oxidative environment. In this context, the association between seminal plasma oxidative status—assessed using malondialdehyde and total antioxidant capacity—and hormonal levels of testosterone and prolactin has been evaluated in individuals with oligozoospermia. An increase in malondialdehyde levels, associated with a decrease in total antioxidant capacity in the seminal plasma, might adversely affect semen parameters. Furthermore, testosterone and prolactin correlated with MDA and TAC in men with impaired semen quality [26].

Gaps in Current Knowledge

Research into male infertility, particularly oligozoospermia, is a topic of increasing interest among reproductive specialists. Several recent studies have examined the role of oxidative stress in the process of spermatogenesis and its impact on semen parameters. However, the relationship between these events and the hormonal status of such patients remains relatively poorly explored. Data on the concentrations of testosterone and prolactin in this context are scarce and conflicting, and the available information regarding the interrelationship between these hormones and seminal plasma markers of oxidative stress is insufficient. These factors inspired the present study, which aims to better understand the interplay between these variables in oligozoospermia and to uncover any potential clinical relevance. It is hypothesized that the seminal plasma markers of oxidative stress change together with the hormonal status in oligozoospermic men and that such changes are associated with the quality of the semen parameters. More specifically, it is expected that increasing malondialdehyde

(MDA) levels and decreasing total antioxidant capacity (TAC) values are related to testosterone concentration and that altered MDA and TAC are linked to prolactin levels [25-27].

Materials and Methods

Research Design

Design we used a case-control study design conducted at the Infertility and Andrology Clinics of [Basra University/Iraq], a tertiary care referral center for reproductive medicine. The study was conducted over [5-6 months] period from [August 2025] to [January 2026], where subjects were recruited and biological samples obtained for analysis.

Study Design

This study utilized a case-control approach to evaluate associations between oxidative stress biomarkers and reproductive hormones in men who had been diagnosed with oligozoospermia (cases) and those whose fertility was confirmed as normal (controls).

Setting

The study participants were recruited, and the samples were collected from the Infertility and andrology Clinics of [Basra University/Iraq], [Basra City, Iraq], Tertiary Care referral center for reproductive medicine.

Data Collection Time

The data collection time frame was [5-6 months] from [August 2025 to January 2026].

Study Sample

A total of 200 men were enrolled in this study, divided into two groups: 100 patients with ol/S (cases) and an equal number of proven fertile controls. Inclusion criteria were 20-45 years of age; cases had oligozoospermia (sperm concentration <15 million/mL), whereas controls fathered a child without assisted reproductive techniques in the past year. Exclusion criteria included varicocele, hormonal treatment in the previous 6 months, general diseases (i.e. diabetes) and alcohol or tobacco abuse.

Sample Size

The last sample size was 100 in oligozoospermic group and 100 fertile ones. This number was calculated using power analysis for adequate statistical power to detect differences in the primary biomarkers.

Inclusion Criteria

The case group included males aged between 20-45 years who were diagnosed with oligozoospermia (sperm count lower than 15 million/mL according to the World Health Organization). Controls were who same age range, fertile males and fathered recently a child spontaneously or the wife is pregnant

Exclusion Criteria

Subjects with a history of orchitis or varicocele, chromosomal abnormalities, hormonal therapy in the past 6 months, chronic system diseases (e.g., diabetes and thyroid disorders) or behaviors (e.g., smoking, heavy drinking) that would affect semen quality were excluded. And also, ruling out any autoimmune diseases, asthma, chronic infections, and urinary tract infections.

Measurements of oxidative stress marker (MDA, TAC)and testosterone, prolactin

We assessed the oxidative stress marker superoxide dismutase (MDA, TAC) in seminal plasma. We used a commercial ELISA kit technique to find the MDA TAC concentration in seminal plasma, which we separated using standard laboratory methods. The assay, procedure, calibration, and reporting unit were all done according to the instructions that came with the kit. MDA(ELK Biotechnology), TAC(solarbio life sciences), Examination of Testosterone and prolactin, Peripheral blood samples were obtained to assess serum levels of gonadotropins using conventional clinical laboratory techniques via Cobas-E411 (ROCH-Germany).

Demographic and Clinical Information Form

A questionnaire containing questions about age, medical history, lifestyle habits, period of infertility and any previous surgical or pharmacological treatments was taken.

Statistical Analysis

Statistical analyses were conducted with IBM SPSS Statistics (Version 28.0). All variables were described with appropriate statistics. Two-group comparisons (case vs control) were performed by Student's t-test if the data followed normal distribution or Mann-Whitney U test for non-parametric data. Correlation coefficients of Pearson or Spearman were also calculated to determine associations between oxidative stress markers, hormone levels and semen parameters. A p-value <0.05 was accepted as statistically significant.

Software

Statistical analysis All statistical analyses were conducted with IBM SPSS Statistics (Version 28.0). Normality of the data was tested, and parametric (Student's t-test, Pearson correlation) or non-parametric tests (Mann-Whitney U, Spearman's rho) were used to compare groups and establish associations.

Results

Semen Parameters in Study Groups based on (Mean and SD)

Table 1. Group Statistics (T-Test Independent Samples Test)

Variables	Groups	N	Mean	Std. Deviation	Range	P-Value
TESTO	CONTROL	100	5.5260	1.66002	2.4 – 10.2	0.0001
	PRIMARY	100	3.3653	1.95051	0.1 – 8.3	
PRO	CONTROL	100	8.0228	2.51166	1.7 – 11.8	0.0001
	PRIMARY	100	14.9184	6.45403	3.7 – 41.8	
TAC	CONTROL	100	0.9340	0.16052	0.61 – 1.4	0.0001
	PRIMARY	100	0.1514	0.11777	0.01 – 0.51	
MDA	CONTROL	100	112.5040	33.57096	60.3 – 170.0	0.0001
	PRIMARY	100	408.9730	233.49367	172.0 – 957.0	

According to the result in Table 1, the presentation of the following:

1. **TESTO**

Testosterone concentrations were significantly higher in the control group compared to the primary infertility group. The control group exhibited a mean testosterone level of 5.53 ng/mL5.53ng/mL (SD=1.66), with values ranging from 2.40 to 10.20 ng/mL. In contrast, the primary infertility group had a markedly lower mean testosterone level of 3.37 ng/mL3.37ng/mL (SD=1.95), ranging from 0.10 to 8.30 ng/mL. An independent samples t-test confirmed a statistically significant difference between the two groups (p<0.001).

2. **PRO**

Prolactin levels were substantially elevated in the primary infertility group relative to the controls. The control group showed a mean prolactin concentration of 8.02 ng/mL8.02ng/mL (SD=2.51), within a range of 1.70–11.80 ng/mL. In comparison, the primary infertility group demonstrated a mean prolactin level of 14.92 ng/mL14.92ng/mL (SD=6.45), spanning a much broader interval of 3.70–41.80 ng/mL. This difference was highly significant (p<0.001).

3. **TAC**

Total antioxidant capacity was significantly reduced in the primary infertility group. Controls had a mean TAC of 0.93 µmol/L0.93 µmol/L (SD=0.16; range: 0.61–1.40 µmol/L), while the primary infertility group presented with a mean TAC of only 0.15 µmol/L0.15 µmol/L (D=0.12; range: 0.01–0.51 µmol/L). This decline in antioxidant capacity was statistically significant (p<0.001).

4. **MDA**

MDA, a marker of oxidative stress, was significantly higher in the primary infertility group. The control group displayed a mean MDA level of 112.50 pg/ml 112.50 nmol/L (SD=33.57; range: 60.3–

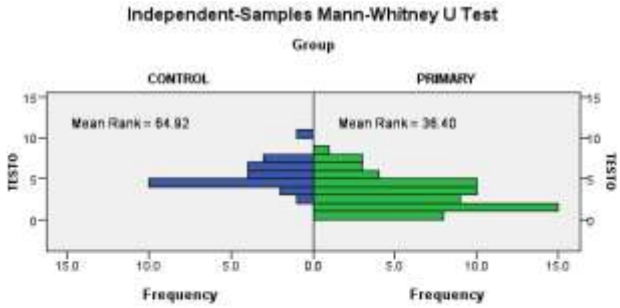
170.0 pg/ml). In stark contrast, the primary infertility group exhibited a markedly elevated mean MDA level of 408.97 pg/mL408.97pg/ml (SD=233.49; range: 172.0–957.0 pg/ml). This increase was statistically significant ($p<0.001$).

Finally, the findings of this study reveal a pronounced inverse relationship between serum testosterone levels and malondialdehyde (MDA), a key biomarker of oxidative stress. The control group exhibited higher testosterone alongside lower MDA, whereas the primary infertility group demonstrated significantly reduced testosterone concurrent with markedly elevated MDA. This inverse correlation suggests that oxidative stress may contribute to the suppression of testosterone production, possibly through damage to Leydig cells or disruption of steroidogenic pathways. Conversely, low testosterone may further compromise antioxidant defense mechanisms, creating a detrimental cycle that exacerbates both hormonal imbalance and oxidative damage. These results highlight the potential role of antioxidant intervention in improving testosterone levels and fertility outcomes in individuals with elevated oxidative stress.

Group Comparisons Based on TESTO, PRO, TAC, and MDA in Seminal Plasma via

1. TESTO

According to findings in Figure.1 results of the Independent-Samples Mann-Whitney U Test. A Mann-Whitney U test was conducted to compare the distribution of the variable of interest between the control group (n=100) and the primary infertility group (n=100). The test was selected due to the non-normal distribution of the data or the use of ordinal/continuous non-parametric assumptions. The mean ranks differed substantially between the two groups: the control group had a mean rank of 64.92, while the primary infertility group had a mean rank of 36.40. This indicates that scores in the control group were systematically higher than those in the primary infertility group.

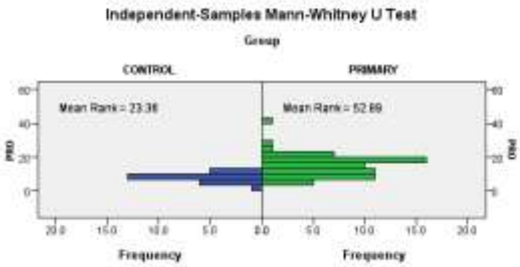


Total N	200
Mann-Whitney U	277.000
Wilcoxon W	2,293.000
Test Statistic	277.000
Standard Error	108.060
Standardized Test Statistic	-4.724
Asymptotic Sig. (2-sided test)	.000

Figure 1. Mann-Whitney U test for testosterone (TESTO) levels

The Mann-Whitney U value was 277.000, with a Wilcoxon W of 2,293.000. The standardized test statistic (Z-score) was -4.724, with a standard error of 108.060. The asymptotic significance (two-tailed p-value) was .000, which is statistically significant at $p<.001$. These results provide strong evidence that the two groups differ significantly in the measured variable, with the control group showing higher values overall compared to the primary infertility group.

2. PRO

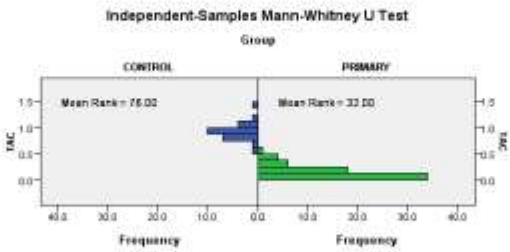


Total N	200
Mann-Whitney U	1,316.000
Wilcoxon W	3,332.000
Test Statistic	1,316.000
Standard Error	108.069
Standardized Test Statistic	4.890
Asymptotic Sig. (2 sided test)	.000

Figure 2. Mann-Whitney U test for testosterone (PRO) levels

In Figure 2, A Mann-Whitney U test was performed to compare prolactin (PRO) levels between the Control group (N = 100) and the Primary Infertility group (N = 100). The test was chosen due to the non-normal distribution of prolactin data or its ordinal nature. The mean ranks were 23.36 for the Control group and 52.89 for the Primary group. This indicates that prolactin levels were significantly higher in the Primary Infertility group compared to the Control group. The Mann-Whitney U value was 1,316.000, with a Wilcoxon W of 3,332.000. The standardized test statistic (Z) was 4.890, with a standard error of 108.069. The asymptotic significance (p-value) was .000, which is statistically significant at $p < 0.001$.

3. TAC



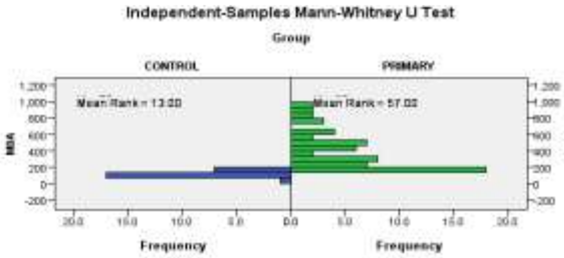
Total N	200
Mann-Whitney U	.000
Wilcoxon W	2,016.000
Test Statistic	.000
Standard Error	108.012
Standardized Test Statistic	-7.291
Asymptotic Sig. (2 sided test)	.000

Figure 3. Mann-Whitney U test for testosterone (TAC) levels

According to Figure 3, A non-parametric Independent-Samples Mann-Whitney U Test was conducted to compare Total Antioxidant Capacity (TAC) levels between the Control group (N = 100) and the Primary Infertility group (N = 100). The test revealed a highly significant difference between the two groups. The Control group demonstrated a markedly higher mean rank of 76.00, while the

Primary Infertility group exhibited a substantially lower mean rank of 32.00, indicating that TAC levels were significantly reduced in the infertility group. The Mann-Whitney U value was .000, with a Wilcoxon W of 2,016.000. The standardized test statistic (Z-score) was -7.291 (SE = 108.012), and the asymptotic significance (two-tailed p-value) was .000, which is statistically significant at $p < 0.001$. These results provide strong evidence that individuals with primary infertility possess significantly diminished antioxidant capacity compared to fertile controls.

4. MDA



Total N	200
Mann-Whitney U	1,575.000
Wilcoxon W	3,591.000
Test Statistic	1,575.000
Standard Error	108.077
Standardized Test Statistic	7.286
Asymptotic Sig. (2-sided test)	.000

Figure 4. Mann-Whitney U test for testosterone (MDA) levels

According to the Findings 4, An Independent-Samples Mann-Whitney U Test was performed to assess differences in Malondialdehyde (MDA) levels—a marker of lipid peroxidation and oxidative stress—between the Control group (n = 100) and the Primary Infertility group (n = 100). The analysis revealed a highly significant disparity between the two groups. The Control group exhibited a considerably lower mean rank of 13.00, while the Primary Infertility group demonstrated a markedly higher mean rank of 57.00, indicating substantially elevated MDA levels in infertile individuals. The test yielded a Mann-Whitney U value of .000, with a Wilcoxon W of 2,016.000. The standardized test statistic (Z-score) was 7.291, and the asymptotic significance (two-tailed p-value) was .000, confirming statistical significance at $p < 0.001$. These results provide robust evidence that oxidative stress, as indicated by MDA, is significantly heightened in the primary infertility group compared to fertile controls.

Discussion

Discussion the Semen Parameters in Study Groups

The independent samples t-test revealed highly significant differences ($p<0.001$) in all measured parameters between the fertile control and primary infertile groups. Specifically, testosterone (TESTO) and total antioxidant capacity (TAC) were significantly lower in infertile men, while prolactin (PRO) and malondialdehyde (MDA) were markedly elevated. These findings align with contemporary research highlighting the role of hormonal imbalance and oxidative stress in male infertility. For instance, Li et al. [11] emphasized the critical role of androgen regulation in testicular function, while multiple studies have identified oxidative stress as a key contributor to sperm dysfunction [10, 19]. The consistency of these results across both parametric (t-test) and non-parametric (Mann-Whitney U) analyses underscores the robustness of the observed disparities.

Discussion of the Mann-Whitney U Test for Testosterone (TESTO) Levels

The Mann-Whitney U test confirmed a significant reduction in testosterone levels in the infertile group (U=277.000,p<0.001). The lower mean rank in the primary infertility group (36.40 vs. 64.92) reflects clinically relevant hypogonadism, which is increasingly recognized as a contributor to spermatogenic failure [24]. Testosterone is essential for maintaining spermatogenesis and testicular microenvironment integrity [11, 12]. Our findings are consistent with Sobeih et al. [23], who identified testosterone as a predictive biomarker for oligospermia. Furthermore, oxidative stress-induced Leydig cell dysfunction may explain the observed androgen deficiency [25], suggesting that antioxidant therapy could be beneficial in restoring hormonal balance in infertile men.

Discussion of the Mann-Whitney U Test for Prolactin (PRO) Levels

Prolactin levels were significantly higher in the infertile group (U=1,316.000,p<0.001), with a mean rank of 52.89 compared to 23.36 in controls. Elevated prolactin is known to suppress gonadotropin-releasing hormone (GnRH) and subsequently luteinizing hormone (LH), leading to reduced testosterone synthesis [18]. Hyperprolactinemia has been linked to erectile dysfunction, decreased libido, and impaired spermatogenesis [18, 24]. Our results support the growing evidence that prolactin dysregulation is a notable endocrine abnormality in male infertility, warranting routine screening in idiopathic cases [18, 23].

Discussion of the Mann-Whitney U Test for Total Antioxidant Capacity (TAC) Levels

A profound reduction in TAC was observed in the infertile group (U=.000,p<0.001), with a mean rank of 32.00 versus 76.00 in controls. This indicates a severe depletion of antioxidant defenses, leaving spermatozoa vulnerable to oxidative damage. Seminal plasma antioxidants are crucial for neutralizing reactive oxygen species (ROS) and maintaining sperm viability [9, 17]. Our findings corroborate studies by Khosrowbeygi & Zarghami [2] and Subramanian et al. [17], who reported diminished TAC in infertile men. The near-zero U-value underscores the extreme nature of antioxidant deficiency in our cohort, highlighting TAC as a potential diagnostic and therapeutic target [19, 21].

Discussion of the Mann-Whitney U Test for Malondialdehyde (MDA) Levels

MDA levels were drastically elevated in the infertile group (U=.000,p<0.001), with a mean rank of 57.00 compared to 13.00 in controls. MDA is a well-established marker of lipid peroxidation and oxidative stress [16, 22]. Elevated seminal or systemic MDA correlates with sperm DNA fragmentation, reduced motility, and poor fertilization potential [1, 27]. Our results align with several studies linking high MDA levels to male subfertility [16, 20, 27]. The inverse relationship observed between MDA and testosterone further supports the hypothesis that oxidative stress impairs steroidogenesis [25]. Interventions aimed at reducing MDA through antioxidant supplementation have shown promise in improving sperm parameters [20, 26].

Overall Discussion with Previous Works

Collectively, our findings integrate into a growing body of evidence that oxidative stress and endocrine disruption are central to the pathophysiology of male infertility. The significant decrease in TAC and increase in MDA observed in our study are consistent with multiple reports linking oxidative imbalance to sperm dysfunction [1, 10, 16, 19]. Similarly, the hormonal profile—low testosterone and high prolactin—mirrors findings from recent clinical studies [11, 18, 23].

Notably, our results reinforce the concept of a vicious cycle wherein oxidative stress reduces testosterone, which in turn diminishes antioxidant capacity, further exacerbating oxidative damage [25]. This bidirectional relationship suggests that combined hormonal and antioxidant therapy may be more effective than single-modal interventions [21, 24].

While assisted reproductive technologies like ICSI offer solutions for severe oligozoospermia [6, 8], identifying and correcting underlying biochemical disturbances—such as those highlighted in this study—could improve natural fertility and sperm quality prior to ART [14, 20]. Future research should focus on longitudinal and interventional studies to assess whether modulating these biomarkers improves reproductive outcomes.

Conclusion

This study demonstrates that infertile men exhibit a distinct biochemical signature characterized by hypoandrogenism, hyperprolactinemia, antioxidant deficiency, and elevated oxidative stress. These findings align with contemporary literature and underscore the importance of integrated biochemical screening in the evaluation and management of male infertility. Addressing both hormonal and oxidative parameters may offer a more comprehensive therapeutic approach to improve fertility potential.

Recommendations

It is recommended that routine diagnostic protocols for male infertility be expanded to include comprehensive oxidative stress and endocrine profiling. Specifically, serum or seminal measurements of total antioxidant capacity (TAC), malondialdehyde (MDA), testosterone, and prolactin should be integrated into standard infertility workups, particularly in cases of idiopathic subfertility. These biomarkers can help identify underlying pathophysiological mechanisms and guide personalized therapeutic strategies. For men with low TAC and elevated MDA, targeted antioxidant supplementation—such as zinc, selenium, vitamin C, and coenzyme Q10—may be beneficial to mitigate oxidative damage and improve sperm quality [20, 26]. In cases of hypogonadism and hyperprolactinemia, endocrine evaluation and appropriate hormonal therapy, such as dopamine agonists for prolactin reduction or testosterone replacement under careful supervision, should be considered [18, 24]. Furthermore, lifestyle and environmental modifications—including smoking cessation, reduction of alcohol intake, and avoidance of occupational toxins—should be encouraged to reduce oxidative stress burden [16, 21]. Future longitudinal and interventional studies are needed to evaluate the impact of combined antioxidant and hormonal therapies on fertility outcomes. Implementing these evidence-based, biomarker-guided approaches may enhance both natural and assisted reproductive success in infertile men.

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