HUE UNIVERSITY
UNIVERSITY OF MEDICINE AND PHARMACY

NGUYEN DAC NGUYEN

EVALUATION OF THE THE IMPACTS OF OXIDATIVE STRESS ON MALE REPRODUCTIVE FUNCTIONS AND THE RESULTS OF ANTIOXIDANT THERAPY

SUMMARY OF THE PH.D. THESIS IN MEDICINE

HUE – 2025

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Academic Supervisors:
Assoc. Professor. PhD. MD. LE MINH TAM
Professor. PhD. MD. CAO NGOC THANH

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Academic Supervisors:
Assoc. Professor. PhD. MD. LE MINH TAM
Professor. PhD. MD. CAO NGOC THANH

Examiner 1: Assoc. Professor. PhD. MD. LE HOANG

Examiner 2: Assoc. Professor. PhD. MD. VU VAN DU

Examiner 3: Assoc. Professor. PhD. MD. TRAN THUA NGUYEN

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I. INTRODUCTION

Infertility is an increasingly prominent problem among couples during their reproductive ages. Numerous reports have shown that male factor involvement occurs in more than half of infertility cases, therefore, the assessment of male reproductive function has drawn growing attention in recent times. Over the past two decades, many studies on sperm function, especially sperm DNA fragmentation and oxidative stress in semen, have been conducted to further evaluate male reproductive dysfunction.

Oxidative stress is used to describe an imbalance of the body's oxidative state due to high levels of reactive oxygen species or low levels of antioxidants. This condition affects not only reproductive function but also the efficacy of infertility treatment, as shown by a decrease in the following indicators: fertilization rate, biochemical pregnancy rate and clinical pregnancy rate. Evaluation of oxidative stress and sperm reactive oxygen species will provide additional information on sperm function and sperm DNA integrity. Therefore, diagnosing oxidative stress and supporting treatment with antioxidant therapies is one of the potential strategies in infertility treatment, especially in case of male infertility.

Antioxidant therapy, which is increasingly utilized in infertility treatment centers, has been proved in several studies to be effient in improving semen analysis parameters, reducing sperm DNA fragmentation and increasing the patient's ability to conceive. However, certain obstacles in applying this therapy include but not limit to the standardization of treatment regimens, subjects, treatment duration. Because of random variations in intervention methods and sample sizes that are not strong enough to draw final conclusions, the effects of antioxidant therapy in men on the final pregnancy outcome have not yet been confirmed. Meanwhile, almost no domestic studies have been conducted to evaluate the effects of oxidative stress on sperm quality through the method of measuring the oxidation-reduction potential.

Based on the urgency and importance of improving the effectiveness of infertility treatment, especially for oxidative stress, we decided to conduct the thesis: " EVALUATION OF THE THE IMPACTS OF OXIDATIVE STRESS ON MALE REPRODUCTIVE FUNCTIONS AND THE RESULTS OF ANTIOXIDANT THERAPY" with the following 2 objectives:

1. Investigate the effects of oxidative stress on sperm quality based on semen analysis results and sperm DNA fragmentation in infertility cases.

2. Evaluate the results of antioxidant therapy on some indicators of sperm quality.

2. SCIENTIFIC SIGNIFICANCE AND CONTRIBUTIONS OF THE THESIS

Semen oxidative stress is a prevalent abnormality among infertile men and causes a marked decline in male reproductive function as shown by indicators such as semen analysis, sperm DNA stability, and sperm function. Many risk factors have been found to be associated with oxidative stress in semen such as: radiation, high temperature, toxins, diet, varicocele, metabolic disorders, orchitis, etc. Controlling risk factors is one of the treatment approaches to promote sperm quality. In addition, the use of antioxidants is promising to pose many positive effects on male reproductive function. The results of this study have made practical contributions in confirming the adverse effects of seminal oxidative stress on sperm quality, and the effects of antioxidant therapy on sperm quality indicators.

Scientific significance: The study has presented the characteristics of semen oxidative stress in the male population from infertile couples, and determined the effects of oxidative stress on sperm quality through indicators such as semen analysis and sperm DNA fragmentation. In addition, the study showed advantageous outcomes after using antioxidants proved by sperm quality indicators,and sexual function in men. This further strengthens the scientific evidence for the diagnosis and treatment of semen oxidative stress in infertile men.

Practical significance: This thesis provides a basis for the recommendation towards the application of semen oxidative stress assessment in cases of male infertility and the use of antioxidants to enhance sperm quality. It also provides evidence of the adverse effects of oxidative stress on sperm quality, some risk factors that can affect sperm quality through the mechanism of oxidative stress as well as the evidence for the effects of oxidative stress on sexual function in men.

3. THESIS LAYOUT

The thesis has 140 pages including an introduction with 2 pages, literature review with 38 pages, subjects and methodology with 30 pages, results with 36 pages, discussion 31 pages, conclusion with 2 pages and 1 page of recommendation.

The thesis contains 56 tables, 18 figures, 1 diagram, 08 charts, 173 references including 11 Vietnamese documents and 162 English documents.

Chapter I: LITERATURE OVERVIEW

1.1. SPERMIOGENESIS

1.2. MALE REPRODUCTIVE FUNCTION

1.3. INTRODUCTION OF OXIDATIVE STRESS

1.3.1. Definition

Oxidative stress is a state of imbalance between oxidant radicals and biological barriers of antioxidants to ensure the physiological activities of the body.

1.3.2. Origin of oxidative stress

The effects of OS are intensified by the decline of cellular protection mechanisms and many potential factors that can lead to increased ROS include: endogenous factors (from gametes and the environment or interventions on embryos/gametes) and exogenous factors (in vitro factors such as culture medium, partial pressure of oxygen).

1.3.2.1. Origin of oxidative radicals in sperm

1.3.2.2. Oxidative stress – inducing diseases

Some diseases which are related to oxidative stress include: Varicocele, testicular cancer, obesity, infections, erectile dysfunction.

1.3.3. Risk factors of oxidative stress

1.3.3.1. External factors

1.3.3.2. Assisted reproduction – related factors

1.3.4. Pathogenesis

1.3.4.1. Molecular biological effects

Oxidative stress has a pathogenesis mechanism including: lipid peroxidation, sperm DNA damage, increased apoptosis and mitochondrial dysfunction, thereby causing genetic adjustments.

1.4. DIAGNOSIS OF OXIDATIVE STRESS

1.4.1. Current oxidative stress tests

1.4.2. Some popular OS testing techniques

1.4.2.1. Measurement of ROS by photochemical method

1.4.2.2. Measurement of total antioxidant capacity in semen (TAC)

1.4.2.3. Measurement of OS by determining the level of oxidation-reduction potential (ORP)

ORP is a recent direct method of testing OS that assesses the balance between oxidation and reduction potential in biological samples. This technique is performed based on the Oxidative Potential Measurement System in Male Infertility Patients (Male Infertility Oxidative System - MiOXSYS, Aytu BioScience Inc., Englewood, CO, USA). This system includes the MiOXSYS analyzer and sensors.

According to Argawal (2019), ORP of above 1.37 mV/10^6 sperm/mL is concluded to increase OS. Unlike other tests or markers, ORP can be used in the future as an independent method to assess sperm quality in male infertility patients.

Gill et al. determined the cut-off of ORP value to distinguish infertile and functional men of 1.40 mV/million sperm/mL (AUC = 0.857).

In 2019, Homa et al. found an inverse relationship between total reactive oxygen species, ORP, DFI with motile sperm percentage ($p = 0.0012$; 0.0002; <0.0001) and viable sperm percentage ($p < 0.0001$; 0.019; <0.0001).

1.5. INTERVENTION THERAPY

1.5.1. Antioxidants

Antioxidants include enzymatic antioxidants (SOD, glutathione reductase - GSH, catalase, etc.) and non-enzymatic antioxidants (vitamin C, vitamin E, folic acid, L-Carnitine, Coenzyme Q10, and melatonin).

1.5.1.1. Enzymatic antioxidants

1.5.1.2. Non-enzymatic antioxidants

Non-enzymatic antioxidants include synthetic antioxidants or micronutrients that inhibit ROS production, such as vitamin E, vitamin C, melatonin, and other natural antioxidants.

1.5.2. Other lifestyle-related interventions

Current evidence suggests that sperm quality in men can be improved when obesity is controlled through exercise or dietary changes. Although BMI may not change significantly, sperm parameters such as concentration, motility, normal morphology and DNA fragmentation all have good improvements.

Quitting smoking completely is very crucial to improve semen parameters, because smoking has been shown to cause deterioration in sperm quality and male reproductive function, as well as the effectiveness of assisted reproductive cycles.

Psychological adjustments are also important, including meditation and yoga, which can help increase physical fitness and male sexual activity.

1.6. CURRENT RESEARCH

1.6.1. Studies in the world

- According to Alahmar (2019), an increase in oxidative radicals along with a decrease in antioxidant capacity will lead to OS, leading to lipid peroxidation of sperm membrane, reduced motility, sperm DNA damage, significantly affecting pregnancy rate and assisted reproductive results, and increasing the risk of genetic diseases in future generations.

- Tunc (2009) found that men with increased DNA fragmentation who were treated with antioxidants before IVF-ICSI would help increase sperm DNA stability and reduce the production of oxidative radicals in semen.

- Another report by Ross (2010) et al. analyzed 17 randomized trials, including a total of 1665 cases of male infertility, and concluded that the use of oral antioxidants in infertile men may have beneficial effects on sperm quality and pregnancy rates.

- A 2019 Cochrane review of 48 RCTs with a total of 4179 infertile men compared pregnancy rates, miscarriage rates, some semen parameters, and sperm DNA fragmentation. They concluded that the current evidence is based on randomized controlled trials with very small sample sizes. The effectiveness of antioxidants clearly improves reproductive function. However, the results are inconsistent because of variation in treatment regimens, methods of selecting assessment indicators, and other confounding factors.

- According to a 2022 Cochrane review, weak evidence from 12 randomized controlled trials suggests that antioxidant supplementation in infertile men may improve live birth rates for couples attending fertility clinics.

1.6.2. Studies in Vietnam

- A study of Huynh Thi Hong Vinh (2013) evaluated the oxidative stress status in semen of infertile men by photochemical method and found that the ROS index was inversely correlated with sperm concentration ($R=-0.123$, $p<0.01$) and sperm motility ($R=-0.166$, $p<0.01$), and the lowest ROS concentration was found in the semen samples of the group of patients with normal semen index.

- Similarly, Bach Huy Anh (2024) concluded that high levels of oxidative stress in semen were associated with a decrease in total sperm count in the group of infertile patients. This study additionally detected gene clusters that caused oxidative stress in semen.

CHAPTER 2
SUBJECTS AND METHODOLOGY

2.1. RESEARCH SUBJECTS AND LOCATION

2.1.1. Research subjects

The study includes male participants from couples diagnosed with infertility according to the World Health Organization (WHO) criteria, who sought diagnosis and treatment at the Reproductive Endocrinology and Infertility Center, University of Medicine and Pharmacy, Hue University, between December 2020 and December 2023.

2.1.2. Selection criteria

Objective 1: male participants of infertile couples satisfying the following criteria:

- Semen sample obtained by masturbation.
- Semen sample eligible for semen analysis, test for sperm DNA fragmentation, and seminal oxidative stress.
- Complete research information according to the survey form.
- Agree to participate in the study.

Objective 2: Patients in the research who agree with the given treatment course and have sperm abnormalities, satisfying at least 1 of the following 3 criteria:

- Abnormal semen analysis results
- Sperm DNA fragmentation determined by the chromatin dispersion method.
- Increased ORP in the semen sample.

2.1.3. Exclusion criteria

- Patients with ongoing acute and chronic systemic diseases (acute genitourinary infections, cirrhosis, renal failure, malignancy, etc.).
- History of treatment with antioxidant drugs within the last 6 months.
- Retrograde ejaculation.
- Azoospermia.
- Patients who have stopped treatment or unable to follow up.

2.2. METHODOLOGY

2.2.1. Study design

- **Objective 1:** Cross-sectional descriptive study
- **Objective 2:** Longitudinal, interventional study

2.2.2. Sample size

Objective 1:

The sample size of men in infertile couples assessed for oxidative stress was calculated using the formula for estimating sample size based on the prevalence of the disease:

$$N = Z^2_{1-\alpha/2}P(1 - P)/d^2$$

The estimated sample size was: 325 cases.

The actual sample size of men in infertile couples that we collected included 351 cases.

Objective 2:

The sample size of men assigned to antioxidant therapy and evaluated for efficacy before and after treatment was calculated using the formula:

$$N = 2C(1 – r)/ES^2$$

The estimated sample size was: 77 cases

The actual number of participants who satisfied research criteria for objective 2 was 84 cases.

2.2.3. Study protocol

Objective 1:

Step 1: Exploit personal information and medical history

- Patient's age, occupation, geographical characteristics.
- Previous smoking habits.
- Habits of drinking alcoholic beverages.
- History of related medical and surgical diseases.

Step 2: Evaluate the husband's clinical characteristics

Determine body mass index

- Height (cm)
- Weight (kg)
- Determine BMI according to the formula:

BMI = Weight (kg) / Height $(m)^2$

- Measure waist and hip circumference

Measure blood pressure

Examine male genitalia

Men are examined for the external genitalia in an upright position, with both arms relaxed. The following structures are evaluated in order: Penis, scrotum, bilateral testicles, bilateral epididymis, bilateral spermatic veins.

Assess Male Sexual Function

Assess the patient's overall sexual function using the International Index of Erectile Function (IIEF)-15, a 15-item scale that assesses five areas related to erectile function and sexual activity.

Step 3. Paraclinical assessment

Biochemical blood test

- Fasting is required before evaluating the lipid profile including total cholesterol, triglycerides, HDL-Cholesterol, LDL-Cholesterol, and blood glucose.

Evaluation of male reproductive function

**Male reproductive hormones*

- FSH, LH, prolactin, and total testosterone are quantified.

**Scrotal ultrasound*

- Scrotal ultrasound is used to assess the volume of bilateral testicles, testicular density, and ultrasound homogeneity; The presence of varicocele is assessed by color Doppler ultrasound; Resistance index (RI), peak systolic velocity (PSV, m/s), and end-diastolic velocity index (EDV, m/s) of the central branch artery of the testicle are measured by pulse Doppler.

** Semen Analysis*

Table 2.1. Semen Analysis Evaluation

Parameter	**Normal Value (WHO 2010)**
Volume	≥ 1.5 mL
pH	≥ 7.2
Vitality	≥ 58% live sperm
Motility	≥ 32% progressive motile sperm
Sperm Concentration	≥ 15 x 10^6 sperm/mL
Sperm Morphology	≥ 4% sperm with normal morphology
Leukocytes	≤ 1 x 10^6/mL

**Analysis of sperm DNA fragmentation by chromatin diffusion method*

Sperm DNA fragmentation was assessed using the Halosperm® kit (Halotech DNA SL, Madrid, Spain) and its variants.

A DFI of ≤15% is considered normal, patients with a DFI from 15% to 30% had a mild level of sperm DNA fragmentation and those with a DFI of > 30% had a severe level of DNA fragmentation and low fertility.

* *Assessment of oxidative stress by measuring the redox potential:*

The MiOXSYS system was used to assess the static oxidation-reduction potential (sORP) and measure oxidative stress in a semen sample.

- The normal ORP cutoff is 1.34 mV/1 million sperm/mL based on a large-sample multicenter study by Agarwal et al. in 2019:

+ ORP > 1.34 mV/1 million sperm/mL is determined to have oxidative stress.

+ ORP ≤ 1.34 mV/1 million sperm/mL is determined to have no oxidative stress.

Objective 2:

Step 4: Intervention by antioxidants

- Male patients from infertile couples who meet treatment criteria were consulted to be treated by antioxidant regimen.

- Profortil (abbott, USA) was utilized in the treatment regimen with a dose of 2 capsules/ day within 3 months.

Step 5: Monitor intervention outcomes

- After 3 months of treatment with the antioxidant regimen, the patient will be re-examined.

- Evaluate the characteristics of sexual activity through the IIEF-15 questionnaire after treatment.

- Perform the semen analysis method after treatment.

- Perform the DNA fragmentation assessment method using the chromatin diffusion method after treatment.

- Perform the oxidative stress assessment method in the semen sample using the oxidation-reduction potential balance measurement method after treatment.

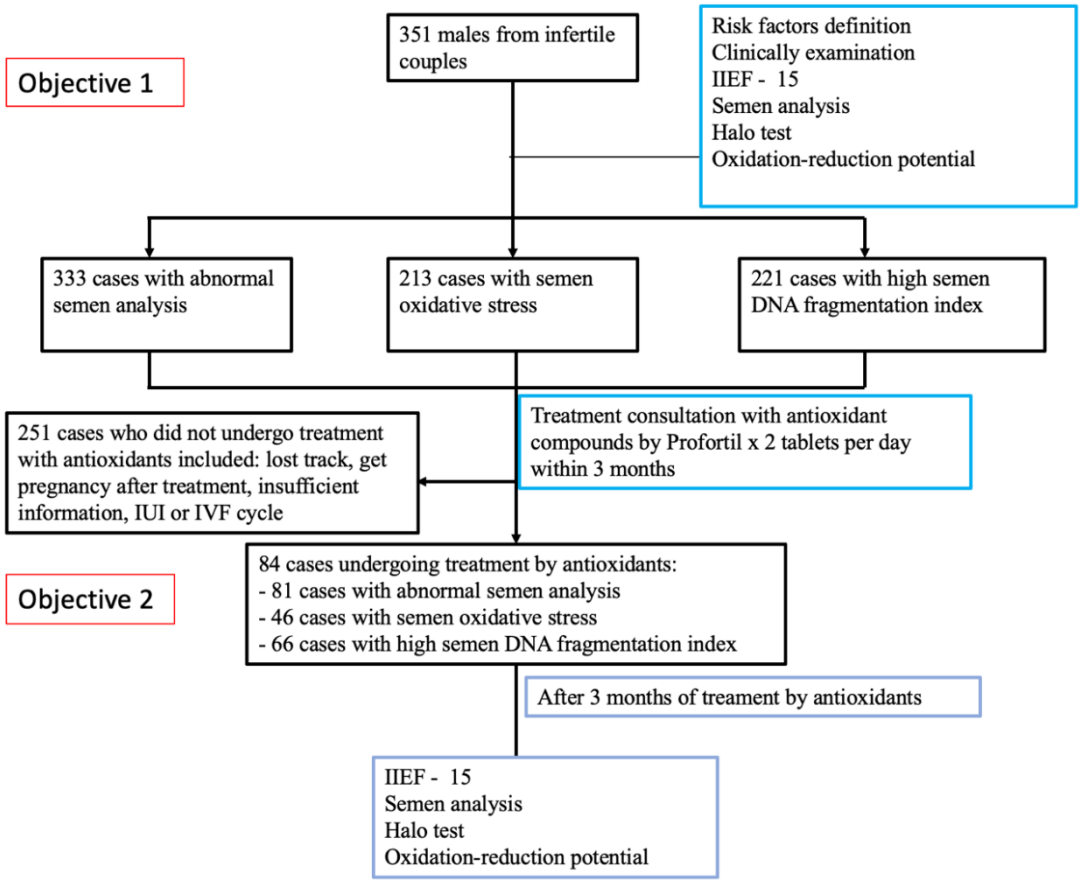

Diagram 2.1. The research flow chart

2.2.4. Research equipment

Clinical assessment equipment

- Printed study form.
- Bench scale with height gauge
- Omron automatic blood pressure monitor (HEM-7117-AP), from Omron Healthcare, Kyoto, Japan.
- Tape measure with centimeter divisions.
- Prader ruler.

Clinical testing equipment

- Cobas E immunoassay analyzer (Cobas 6000/8000) from Roche Diagnostic GmbH.
- Roche/Cobas C assay analyzer (Cobas 6000/8000 Module) from Roche Diagnostics, Indianapolis, USA.
- Microscope, sperm counting chamber.
- Centrifuge, incubator at 37oC and 5% CO2.
- Halosperm® kit, Halotech DNA SL, Madrid, Spain.

- Agarose 1%.

- Denaturant Agent (DA): hydrochloric acid solution (HCl 0.08 N).

- Lysis Solution (LS): contains Dithiothreitol and Triton X-100.

- Distilled water, 70% ethanol, 100% ethanol

- Optical microscope, refrigerator 4ºC, incubator 95ºC

- Glass slides coated with 0.65% agarose; coverslips (24 x 24 mm); staining plates or trays

- MiOXSYS Oxidation-Reduction potential balance system, CaerusBiotech, Geneva, Switzerland.

- X12 PRO automatic sperm analyzer (Lenshooke, Taiwan).

2.3. RESEARCH VARIABLES

2.4. DATA ANALYSIS

The data were processed using SPSS software version 26 (IBM SPSS Statistics for Windows, Version 26.0) and GraphPad software.

Statistical Methods:

– Normal distribution was tested using the Kolmogorov-Smirnov test.

– Quantitative variables were expressed as mean ± standard deviation if normally distributed, or median (range) if not normally distributed.

– The difference between two means was compared using the independent t-test (for normally distributed data) or the Mann-Whitney U test (for non-normally distributed data).

– The difference in means before and after treatment for data with normal or approximately normal distribution was tested using the paired t-test. If not normally distributed, the Wilcoxon test was used instead, with a significance level of $\alpha = 0.05$.

– Diagnostic test evaluation: ROC curve analysis using the area under the ROC curve (AUC) to assess the ORP value for distinguishing cases with abnormal semen analysis results or sperm DNA fragmentation.

2.5. RESEARCH ETHICS

- The study was approved by the Biomedical Research Ethics Committee, Hue University of Medicine and Pharmacy, Hue University, no H2021/390.

- The study was registered as a clinical trial with code NCT04509583.

CHAPTER 3:
RESULTS

Through this study, we collected data on 351 male patients who met the selection criteria and were treated at the Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy Hospital.

3.1. CHARACTERISTICS OF THE STUDY SAMPLE

3.1.1. General characteristics of the study subjects

3.1.2 Clinical characteristics of the study group

3.1.2.1. Anthropometric characteristics

Table 3.4. Anthropometric characteristics (N=351)

Characteristic	Mean ± SD	Median	Lowest	Highest
Height m)	1.68 ± 0.05	1.68	1.53	1.83
Weight (kg)	65.89 ± 8.83	65.00	50	90
Waist circumference (cm)	85.34 ± 8.33	86.00	62	110
Hip circumference (cm)	96.43 ± 5.82	96.00	73	115
Waist-to-hip ratio	0.88 ± 0.06	0.89	0.65	1,13
Systolic BP (mmHg)	118.75 ± 11.67	120.00	90	170
Diastolic BP (mmHg)	75.88 ± 9.87	80.00	60	120
BMI (kg/m^2)	23.34 ± 2.71	23.39	16.85	32.69

The mean height was 1.68 ± 0.05m, the mean weight was 65.89 ± 8.83 kg. BMI in the study sample was 23.34 ± 2.71 kg/m^2

3.1.2.2. Testicular characteristics: location, density, volume (Prader orchidometer)

Table 3.5. Characteristics of genitalia (N=351)

Characteristics		Number	Percentage (%)
Testicular density	Normal	341	97.2
	Abnormal	10	2.8
Left testicular volume (mL)	Mean ± SD	10.38 ± 3.41 (4-30)	
Left testicular volume (mL)	Mean ± SD	10.57 ± 3.48 (0-30)	
Epididymal density	Normal	340	96.9

	Abnormal	11	3.1
Epididymal volume	Normal	338	96.3
	Abnormal	13	3.7
Phimosis		5	1.4
Cryptorchidism		2	0.6
Palpable tumour		8	2.3
Urethral stricture		1	0.3
Varicocele		81	25.9
Varicocele grading	Grade 1	66	18.8
	Grade 2	16	4.5
	Grade 3	9	2.6

Left and right testicles are of equivalent volumes, with 10.38 ± 3.41 mL và 10.57 ± 3.48 mL respectively. There are 25.9% patients who were diagnosed with varicocele, among whom the majority was classified as mild with a proportion of 18.8%.

3.1.2.4. Severity of erectile dysfunction according to IIEF

Table 3.6. Severity of erectile dysfunction according to IIEF (N=351)

Characteristic	**Number**	**Percentage (%)**
No dysfunction	257	73.2
Mild dysfunction	88	25.1
Moderate dysfunction	4	1.1
Severe dysfunction	2	0.6
Total	351	100.0
IIEF score	62.49 ± 9.54 (16-75)	

According to IIEF, there are 73.2% cases defined as normally functional, 25.1% patients with mild dysfunction, 1.1% and 0.6% cases with moderate and severe dysfunction, respectively.

3.1.3. Paraclinical parameters

Table 3.9. Paraclinical parameters(N=351)

Characteristic	Median	Mean ± SD	Lowest	Highest
FSH (mIU/mL)	4.74	5.30 ± 2.74	1.02	22.51
LH (mIU/mL)	5.24	5.79 ± 2.91	1.60	31.76
Testosterone (ng/mL)	4.47	4.78 ± 2.75	1.01	40.94
Prolactin (mIU/mL)	243.10	294.32 ± 203.77	78.76	2784.00
Glucose huyết thanh (mmol/L)	5.38	5.59 ± 1.06	3.90	15.60
Cholesterol TP (mmol/L)	5.00	5.08 ± 0.90	2.95	8.53
LDL (mmol/L)	3.21	3.24 ± 0.84	0.59	6.12
HDL (mmol/L)	1.23	1.26 ± 0.27	0.65	2.22
Triglycerid (mmol/L)	1.88	2.29 ± 1.57	0.55	15.60

Mean FSH in our study was 5.30 ± 2.74 mIU/mL, while that of LH was 5.79 ± 2.91 mIU/mL, average testosterone level was measured at 4.78 ± 2.75 ng/mL. Other biochemical blood parameters stayed relatively within normal ranges.

3.2. INVESTIGATE THE EFFECTS OF OXIDATIVE STRESS ON SPERM QUALITY BASED ON SEMEN ANALYSIS RESULTS AND SPERM DNA FRAGMENTATION IN INFERTILITY CASES

3.2.1. Oxidative stress characteristics in semen and sperm quality

Table 3.11. Oxidative stress severity based on ORP (N=351)

Characteristic	Number	Percentage (%)
ORP > 1.34 mV/1 million sperm/mL	213	60.7
ORP ≤ 1.34 mV/1 million sperm/mL	138	39.3
Mean ± SD Median (Lowest – Highest)	1.79 ± 2.44; 1.08 (0.09-25.82)	

There are 213 cases, which took up 60.7%, with an ORP of more than 1.34 mV/1 million sperm/mL, the remaining cases (39.3%) with a result of lower than the cutoff ORP level. The mean The mean value of the redox potential balance was 1.79 ± 2.44 mV/million sperm/mL.

3.2.2. Association between some common characteristics and oxidative stress

3.2.3. Association between some clinical characteristics and oxidative stress

3.2.3.3. Association between detected pathologies and oxidative stress

Table 3.18. Association between genital abnormalities and oxidative stress (N=351)

Characteristic		Oxidative stress				p
		No		Yes		
		N	%	N	%	
Varicocele	**No**	**167**	**64.2**	**93**	**35.8**	**0.021**
	Yes	**46**	**50.5**	**45**	**49.5**	
Phimosis	No	210	60.7	136	39.3	1.00
	Yes	3	60.0	2	40.0	
Erectile dysfunction	No	163	63.4	94	36.6	0.082
	Yes	50	53.2	44	46.8	

Patients with varicocele had a high ORP rate of 49.5%, higher than the group without varicocele (35.8%), p=0.021. There is no difference in the rate of high ORP observed among patients with other disorders such as erectile dysfunction and phimosis with p=0.082 and p=1.00.

3.2.4. Association between paraclinical parameters and oxidative stress

3.2.4.1. Association between semen analysis results and oxidative stress

Table 3.21. Association between semen analysis results and oxidative stress (N=351)

Characteristic		Oxidative stress				p
		No		Yes		
		N	%	N	%	
Volume	Normal	194	59.7	131	40.3	0.179
	Abnormal	19	73.1	7	26.9	
pH	**Normal**	**200**	**59.5**	**136**	**40.5**	**0.035**
	Abnormal	**13**	**86.7**	**2**	**13.3**	
Vitality	Normal	210	60.7	136	39.3	0.975
	Abnormal	3	60.0	2	40.0	
Motility	Normal	27	58.7	19	41.3	0.767
	Abnormal	186	61.0	119	39.0	
Concentration	**Normal**	**199**	**71.6**	**79**	**28.4**	**<0.001**

	Abnormal	**14**	**19.2**	**59**	**80.8**	
Normal morphology	Normal	33	63.5	19	36.5	0.657
	Abnormal	180	60.2	119	39.8	
Head abnormalities (X ± SD)		**96.3 ± 2.06**		**96.8 ± 2.22**		**0.029**
Midpiece – tail abnormalities (X ± SD)		**47.12± 6.26**		**50.24 ± 7.36**		**<0.001**
Semen analysis results	Normal	9	50.0	9	50.0	0.341
	Abnormal	204	61.3	129	38.7	

Low sperm concentration was associated with a significantly higher rate of oxidative stress than the group with normal sperm concentration (80.8% vs. 28.4%, $p < 0.001$). The group with oxidative stress had a higher rate of head abnormalities with 96.8% compared to a rate of 96.3% in the group without oxidative stress ($p = 0.032$). The rate of midpiece-tail abnormalities was higher in the oxidative stress group with 50.24% compared to its counterpart group with 47.12% ($p < 0.001$).

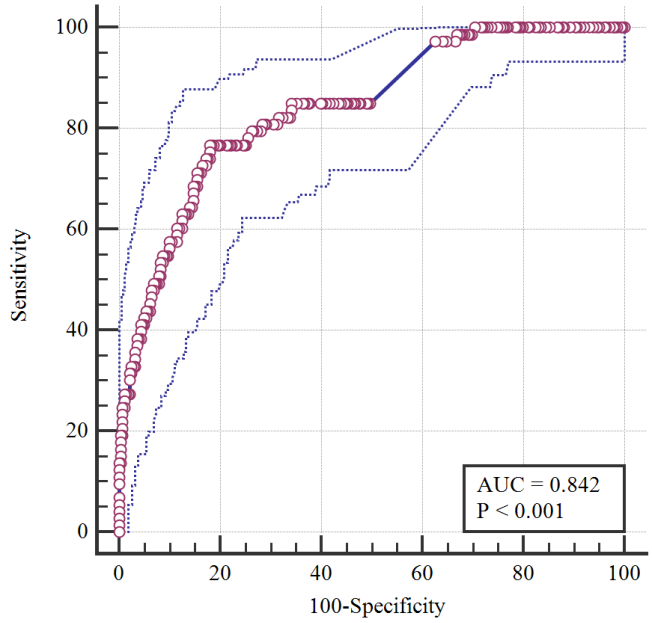

Figure 3.3. ORP cutoff in discriminating abnormal sperm concentration

Table 3.23. Value under the ROC curve of ORP in predicting abnormal sperm concentration

Factor	AUC (95% CI)	Cutoff	Sensitivity(%)	Specificity(%)	p
ORP (mV/1 million sperm/mL)	0.84 (0.80 – 0.88)	1.62	82.20	66.20	<0.001

The ROC curve determined the cut-off of the ORP value of 1.62 mV/million sperm/mL to distinguish cases with abnormal sperm concentration with AUC: 0.84 (0.80 - 0.88), p<0.001; its sensitivity was 82.20% and its specificity was 66.20%.

3.2.4.2. Correlation between Halosperm test results and oxidative stress

Table 3.24. Correlation between halosperm test results and ORP (N=351)

Halosperm	Without oxidative stress			With oxidative stress			p
	Median	Smallest	Highest	Median	Smallest	Highest	
Big halo	78.00	7	391	99.00	0	435	0.066
Medium halo	**318.00**	**74**	**418**	**287.50**	**21**	**421**	**<0.001**
Small halo	49.00	13	167	53.00	10	243	0.225
No halo	17.00	3	138	18.00	2	162	0.517
Degraded	16.00	2	103	16.50	0	83	0.676
DFI (%)	17.20	6.20	63.20	17.80	3.00	68.80	0.188

The total number of sperm with big halos in the group with oxidative stress in semen was similar to that of its counterpart without oxidative stress (99.00 vs. 78.00, p = 0.066). The DNA fragmentation index in the group without oxidative stress was equivalent to that in the group with oxidative stress (p = 0.188).

3.3. EVALUATE THE RESULTS OF ANTIOXIDANT THERAPY ON SOME INDICATORS OF SPERM QUALITY

In this study, antioxidant regimen was applied to 84 patients who were later divided into subgroups based on sperm quality characteristics to evaluate the effectiveness of the intervention as follows: group with abnormal semen analysis results (N1 = 81), group with high ORP index (N2 = 46), and group with high DNA fragmentation results (N3 = 66).

3.3.1. Evaluation of treatment effectiveness in the group with abnormal semen analysis results (N=81)

3.3.1.1. Treatment effectiveness on erectile dysfunction

Table 3.29. Erectile dysfunction score according to IIEF scale before and after treatment (N=81)

IIEF score	**Before intervention**	**After intervention**	Difference (TB±SEM)	**p *Wilcoxon Test***
Total	**63.15±9.30**	**66.36±7.77**	**-3.21±0.35**	**<0.001**
Erectile function	25.14±4.44	26.40±3.76	-1.26±0.19	<0.001
Intercourse satisfaction	12.28±2.59	13.11±2.21	-0.83±0.13	<0.001
Orgasmic function	9.17±1.34	9.23±1.25	-0.06±0.08	0.456
Sexual desire	8.30±1.62	8.30±1.39	-0.53±0.11	<0.001
Overall satisfaction	8.26±1.78	8.79±1.38	-0.53±0.10	<0.001

Patients who had abnormal semen analysis results were noticed to experience improvements in their sexual function according to IIEF-15 scale.

3.3.1.2. Semen analysis

Table 3.30. Semen analysis before and after treatment (N=81)

Semen parameter	**Before intervention**	**After intervention**	Difference (TB±SEM)	**p *Wilcoxon Test***
Volume (ml)	2.78±1.21	2.74±1.07	0.04 ± 0.14	0.805
pH	7.80±0.34	7.80 ± 0.27	0.0±0.04	0.521
Concentration (million sperm/mL)	26.26 ± 18.15	27.02±15.88	-0.77 ±1.69	0.241
Vitality(%)	83.10±7.94	82.43±9.96	0.67±0.94	0.559
Normal morphology(%)	**1.72±1.14**	**2.05±1.28**	**-0.33±0.14**	**0.028**
Motility (%)	**14.93±8.85**	**17.15±8.74**	**-2.22±0.97**	**0.012**

There is a noticeble improvement in the rate of sperm with normal morphology after intervention (2.05 ± 1.28% after treatment compared to 1.72 ± 1.14 % before treatment, p =

0.028). A similar trend was witnessed after treatment regarding motility, with a rate of 14.93% pre-treatment rising to 17.15% post-treatment, p = 0.012.

3.3.1.3. Halosperm test results and ORP

Table 3.31. Halosperm result before and after treatment (N=81)

Semen analysis	**Before intervention**	**After intervention**	**Difference** (TB±SEM)	**p** ***Wilcoxon Test***
Big halo	83.52±74.14	118.07±81.23	-34.56±11.98	0.002
Medium halo	281.79±68.52	288.48±70.93	-6.69±10.15	0.439
Small halo	74.15±42.74	52.52±27.20	21.63±4.79	<0.001
No halo	35.43±29.99	20.67±17.77	14.77± 3.25	<0.001
Degraded	25.09±19.04	20.26 ±17.21	4.83±1.86	0.014
DFI (%)	**26.93±13.58**	**18.69±10.54**	**8.24±1.47**	**<0.001**
ORP (mv/million sperm/mL)	**2.64±3.43**	**1.47±1.56**	**1.17±0.29**	**<0.001**

In patients with abnormal semen analysis, sperm DNA fragmentation improved substantially after treatment.

Table 3.32. ROS testing results before and after treatment (N=81)

			Before intervention	**After intervention**	**P**
Oxidative stress	**Yes**	**N**	46	32	**0.009**
		%	56.79	39.51	
	No	**N**	35	49	
		%	43.21	60.49	
	Total		81	81	
% difference (CI 95%)			17.3 (5.5 – 29.0)		

A reduction in semenal oxidative stress rate was observed after a course of treatment with antioxidants.

Table 3.45. Correlation between semen analysis results and treatment outcomes (N=84)

Parameter	Improved DFI	Mean ± SD	Mean Difference	p
Volume	Có	-0.09 ± 1.13	-0.16	0.810
	Không	0.07 ± 1.32		
pH	Có	0.04 ± 0.22	0.08	0.622
	Không	-0.4 ± 0.41		
Motility (a+b)	**Có**	**-4.70 ± 7.92**	**-4.04**	**0.025**
	Không	**-0.67 ± 8.86**		
Concentration	**Có**	**-4.70 ± 16.92**	**-6.69**	**0.032**
	Không	**1.98 ± 14.75**		
Vitality	Có	0.93 ± 5.72	0.52	0.901
	Không	0.40 ± 9.29		
Normal morphology	Có	-0.56 ± 1.28	-0.43	0.096

There is a notable correlation between the improvement of DFI after treatment and that of semen parameters including sperm motility and concentration.

CHAPTER 4
DISCUSSION

4.1. DISCUSSION OF RESEARCH DESIGN AND TECHNIQUES FOR ASSESSING OXIDATIVE STRESS IN SEMEN

4.2. INVESTIGATE THE EFFECTS OF OXIDATIVE STRESS ON SPERM QUALITY BASED ON SEMEN ANALYSIS RESULTS AND SPERM DNA FRAGMENTATION IN INFERTILITY CASES

4.2.3. Paraclinical characteristics

Oxidative stress characteristics: When compared with the cut-off value of ORP to detect cases with abnormal semen analysis results, our findings were classified as increased oxidative stress in semen samples. Some other similar studies using the method of measuring the oxidation-reduction potential balance to examine the oxidative stress in semen have given variable data (**Table 4.2**).

Table 4.2. ORP value in several studies

Study	Study Subjects	Results
Takashi et al. (2019)	Males with normal reproductive function, and those with varicocele	The average ORP value in males with normal reproductive function was 1.14 ± 1.78 mV/1 million sperm/mL; while that of males with varicocele was 4.02 ± 7.56 mV/1 million sperm/mL.
Kazuhisa et al. (2023)	Males from infertile couples indicated for IVF	The average ORP value was 3.4 mV/1 million sperm/mL.
Majzoub et al. (2020)	Infertile males and control group	The average ORP value in the infertile male group was 1.8 mV/1 million sperm/mL, and in the control group was 0.9 mV/1 million sperm/mL.
Sergio et al.	Males with unexplained infertility	The average ORP value was 3.02 ± 9.50 mV/1 million sperm/mL.
Elbardisi et al.	Infertile males	The average ORP value was 2.94 mV/1 million sperm/mL.

4.2.4. Factors Associated with Oxidative Stress

Associated Diseases:

A study by Barradas (2021) on post-pubertal males with varicocele concluded that varicocele increases sperm lipid peroxidation. This is one of the main pathogenic mechanisms resulted from oxidative stress.

4.2.5. The Impact of Oxidative Stress on Sperm Quality

Semen Analysis Results:

According to a recent 2019 review by Alahmar, the increase in reactive oxygen species (ROS) along with a decrease in antioxidant capacity leads to oxidative stress (OS), resulting in sperm membrane lipid peroxidation, reduced motility, sperm DNA damage, which significantly affects pregnancy rates, assisted reproductive technology outcomes, and increases the risk of genetic diseases in subsequent generations.

Gill (2023) observed that men with poor sperm motility had a notably higher risk of sperm DNA fragmentation (SDF > 20%) and sperm oxidative reduction potential (sORP > 1.37) compared to the control group with normally moving sperm. The risk of sperm DNA damage and oxidative stress in men with immotile sperm was 10 times and 6 times higher, respectively, compared to the control group.

Sperm DNA Fragmentation:

A study by Ho (2022) on men infected with genital pathogens such as *Ureaplasma* or *Trichomonas* found that cases of genital inflammation often enhanced the oxidative stress in semen ($p < 0.001$), which in turn led to an increase in sperm DNA fragmentation ($p < 0.001$).

Homa et al. (2019) reached a conclusion that there was an inverse relationship between total oxidative species, ORP, DNA fragmentation index (DFI) with sperm motility ($p = 0.0012$; 0.0002; <0.0001) and sperm viability ($p < 0.0001$; 0.019; <0.0001).

A recent study by Liu (2021) similarly found that genital tract inflammation could cause a rise in semen leukocytes, which is a major source of oxidative stress in semen and contributes to increased sperm DNA fragmentation.

4.3. EVALUATE THE RESULTS OF ANTIOXIDANT THERAPY ON SOME INDICATORS OF SPERM QUALITY

4.3.1 Treatment outcomes based on semen analysis

Ross et al. (2010) analyzed 17 randomized trials and found that semen parameters after antioxidant therapy were reported to improve significantly in 14 out of the 17 trials. Among these, sperm motility showed the most substantial improvement, with a positive outcome in 9/17 different trials.

Rochdi (2024) concluded that sperm concentration improved noticeably after treatment (before treatment 8.67 ± 1.41, after 3 months 12.17 ± 1.91, after 6 months 19.01 ± 0.86, $p < 0.01$); other parameters such as sperm motility and total sperm count also showed improvement with $p < 0.01$.

A recent analysis by Agarwal et al. (2023) on 1307 publications and 45 RCTs involving 4332 patients proposed several observations: the pregnancy rate was significantly higher in patients treated with antioxidants compared to the control group treated with a placebo or untreated.

Recent Cochrane reviews (2019, 2022) have shown effectiveness in improving sperm quality, as indicated by semen analysis parameters. Specifically, sperm concentration, sperm motility, and sperm morphology were the parameters that showed notable improvements.

4.3.2. Intervention Effectiveness Based on Sperm DNA Fragmentation

A Cochrane review conducted in 2019 concluded that antioxidants clearly help improve

reproductive function. Among the parameters, sperm DNA fragmentation showed significant improvement in studies conducted after 3 or 6 months of antioxidant treatment.

A meta-analysis by Noegroho (2022) based on 9 studies evaluated the effectiveness of antioxidant multivitamin therapy on sperm DNA fragmentation and other semen analysis parameters. The nine studies showed a positive reduction in SDF (sperm DNA fragmentation) rates after antioxidant supplementation.

In a study by Martinez-Soto et al. (2016), the authors observed a drop in SDF% from 22.0% ± 2.1% to 9.3% ± 1.3% ($P < 0.01$) after daily supplementation of 1500 mg DHA.

4.3.3. Intervention Effectiveness Based on Seminal Oxidation-Reduction Potential

A study by Chi et al. (2008) found significantly lower ROS levels in sperm after being washed with antioxidants (196~312 rlu) compared to the control sperm group (604 rlu, $P < 0.05$). This study concluded that supplementing sperm preparation media with EDTA or catalase significantly improved overall sperm function parameters by reducing ROS concentration.

Ciftci (2009) demonstrated that NAC (N-acetylcysteine) significantly improved sperm volume, motility, and semen viscosity. After NAC treatment, the total antioxidant capacity in serum was higher, and total peroxide levels and oxidative stress were lower in the NAC-treated group compared to the control group.

Ahmad (2017) indicated that ascorbic acid was effective in reducing heat-induced oxidative stress during sperm preparation in vitro. Ascorbic acid supplement could be beneficial for semen preparation in IUI, IVF, and ICSI procedures.

4.3.4. Intervention Effectiveness Based on Sexual Function Characteristics

According to Zhang et al., long-term consumption of antioxidants in the diet may eliminate oxidative products and improve erectile function.

Another study by Ghamari (2020) evaluated female sexual dysfunction after supplementation with vitamin E and ginseng. Significant changes in total FSFI (Female Sexual Function Index) scores and subgroup scores were observed after antioxidant treatment. However, the supplements only significantly improved sexual desire and sexual satisfaction. This study found no additional benefits of supplementing with vitamin E and ginseng over placebo in enhancing overall sexual function, but this regimen was more effective in improving sexual desire and satisfaction.

CONCLUSION

1. Investigate the effects of oxidative stress on sperm quality based on semen analysis results and sperm DNA fragmentation in infertility cases.

– Oxidative stress was detected in the semen of 60.7% of patients. The average value of the oxidation-reduction potential (ORP) balance was 1.79 ± 2.44 mV/million sperm/mL; median: 1.08 mV/million sperm/mL.

– Seminal oxidative stress was higher in individuals with varicocele.

– ORP values showed a positive correlation with the percentage of sperm with head abnormalities, percentage of sperm with midpiece - tail abnormalities, and a negative correlation with sperm concentration, slow progressive motility, normal sperm morphology, and the number of sperm with a medium halo.

– The ROC curve determined the cutoff tvalue for ORP to differentiate cases with abnormal sperm concentration as 1.62 mV/million sperm/mL, with a sensitivity of 82.20% and specificity of 66.20%.

2. Evaluate the results of antioxidant therapy on some indicators of sperm quality.

– The antioxidant regimen over 3 months improved sperm quality, as demonstrated by several semen analysis parameters: sperm motility, percentage of sperm with normal morphology, and sperm concentration.

– The antioxidant regimen significantly improved sperm DNA stability, as evidenced by an increase in the number of sperm with big halo, together with a reduction in sperm with small halo, sperm with no halo, degenerated sperm, and sperm DNA fragmentation.

– The antioxidant regimen helped reduce oxidative stress in semen, as shown by the reduction in the oxidation-reduction potential balance after treatment.

RECOMMENDATION

1. Oxidation-reduction potential measurements can be used to assess seminal oxidative stress, especially in cases of unexplained male infertility. A 3-month antioxidant regimen can be used to improve sperm quality in men with abnormal semen analysis, increased seminal oxidative stress, and increased sperm DNA fragmentation.

2. It is necessary to continue studies evaluating the status of oxidative stress in semen by the method of seminal oxidation-reduction potential with larger sample sizes and multi-centers, thereby having a threshold of the oxidation-reduction potential to diagnose sperm quality disorders and reproductive dysfunction in men in the Vietnamese population. In addition, it is necessary to focus on clearly identifying risk factors that increase oxidative stress in semen, especially factors related to lifestyle.

3. Further studies are needed to compare different antioxidant regimens in improving sperm quality to optimize infertility treatment, especially in cases of male infertility.

SCIENTIFIC PUBLICATIONS RELATED TO THIS THESIS

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