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Impact of semen oxidative stress on sperm quality: initial results from Vietnam

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Abstract

Objectives: This study aimed to determine the relationship between oxidative stress (OS) measured by the oxidation–reduction potential (ORP) and the results of semen analysis among men from infertile couples.

Methods: This cross-sectional study included 166 men from infertile couples, determined according to the World Health Organization guidelines. The general characteristics, semen analysis, sperm chromatin dispersion assay, and ORP of all subjects were evaluated and analyzed statistically.

Results: Among 166 men from infertile couples, individuals with OS had a significantly higher DNA fragmentation index (DFI) than men without OS (22.37% ± 11.67% vs. 17.98% ± 8.98%). The sperm concentration, total sperm count, motility rate, and normal morphology were negatively correlated, while and an abnormal head and neck–tail were positively correlated with ORP. There was also a positive association between the DFI and OS level. The optimal ORP threshold for determining sperm quality was 0.77 mV/10^6 sperm/mL (sensitivity, 50.4%; specificity, 93.5%; positive predictive value, 52.9%; negative predictive value, 32.3%).

Conclusions: Determining the ORP suggests that OS has an adverse effect on the total sperm count, sperm motility, sperm concentration, morphology, vitality, and DNA fragmentation index.

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Introduction

Male factors, alone or in combination with female factors, are responsible for 30% to 50% of cases of infertility among couples,[1] and age-standardized infertility rates for men worldwide have increased by 0.291% each year.[2] Various factors have been shown to affect spermatogenesis, but the causes and risk factors of male infertility remain unclear. Known reasons for male infertility include varicocele, congenital anomalies of the genital system, genital infections, erectile dysfunction, and endocrine diseases; however, up to 30% of cases are idiopathic.[3] Although semen analysis, as the most frequently used method in nearly all laboratories, typically reflects the overall functioning of the male reproductive organs,[4] semen analysis alone cannot identify potential male fertility accurately.[4] According to the World Health Organization 2010, almost 15% of infertile men have normal semen parameters,[5] and some intracellular variables may be undetectable by standard sperm analysis. Oxidative stress (OS) occurs when there is an imbalance between oxidant and antioxidant agents that protect the physiological functions of the organism. Sperm contains an antioxidant system that helps to protect spermatogenic cells and mature spermatozoa against the damaging effects of OS; however, the increased generation of reactive oxygen species (ROS) under certain circumstances can overwhelm the protective capacity of the antioxidants, resulting in OS.[3]

A previous report from the United States suggested that OS was a major causes of male infertility.[6] Around 30% to 40% of infertile men have high ROS levels in their semen. The lack of an appropriate cytoplasmic enzyme repair system and the high levels of polyunsaturated fatty acids in their plasma membranes make sperm highly susceptible to the effects of OS, leading to reduced physiological functioning and survival, an increase in mid-sperm morphological defects, and decreased mobility.[7]

OS and redox potential imbalance also affect the integrity of sperm DNA. The DNA fragmentation index (DFI) was increased in sperm samples showing OS, as evaluated by ROS and oxidation–reduction potential (ORP).[8] Lewis et al. similarly demonstrated a higher incidence of aberrant DFI in patients with low ascorbic acid levels compared with those with normal or high ascorbic acid levels.[9] In addition, a retrospective investigation in 2022 showed that changes in the seminal oxygen–reduction balance system were directly associated with the pathophysiology of sperm DNA damage.[10] Another study in bulls also found that, with respect to the effect of ROS, the occurrence of dead superoxide anion-positive sperm in bulls with good sperm freezability was higher than that in those with poor freezability (15.72% and 12.00%, respectively; $P = 0.024$).[11] Muhamed et al. highlighted the importance of improving the post-thaw viability and fertility of sperm through enhanced cryopreservation, in light of the effects of ROS.[12]

ROS levels have recently been recommended as a valuable predictor of sperm quality.[7] OS is associated with a longer duration of infertility, poor pregnancy outcomes during treatment cycles, and an increased risk of miscarriage.[13] Additionally, antioxidants such as vitamins E and C, zinc, folic acid, and selenium have enhanced reproductive and sperm functions.[14] OS is critical factor affecting male reproductive capacity, and the concept of male infertility due to OS (male oxidative stress infertility, MOSI) has opened up new research directions.[15]

This study aimed to determine the relationships between OS and the results of semen analysis in infertile cases, to confirm the role of OS in the pathophysiology of male infertility, with the aim of improving the outcome of assisted reproductive therapy.

Materials and methods

Study design

This cross-sectional study was conducted at the Hue Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy Hospital, Vietnam, between November 2020 and November 2021. The study is reported according to the STROBE guidelines.[16] This study was approved by the Hue University of Medicine and Pharmacy Ethics Committee (approval number H2021/390). All participants in this study provided signed informed consent. During data collection, the identities of all patients were concealed. All procedures were performed in compliance with the study's protocols.

The study included men from infertile couples, determined according to the World Health Organization (WHO) (ICD-11)[17] guidelines. Consecutive men from couples diagnosed with infertility were recruited. Men with azoospermia, retrograde ejaculation, infection, acute systemic illnesses, cancer, or impaired hepatic function, or missing data were excluded from the study.

We collected data on the following patients' characteristics, as potential confounding factors: age, type of infertility, and length of abstinence. The participants were divided into groups according to age (over 35 and under 35 years) and type of infertility (primary and secondary infertility). Female partners in infertile couples were assessed for a history of miscarriage. Semen, sperm DNA fragmentation (SDF) (Halosperm test), and ORP were evaluated in all subjects.

In this study, the sample size was determined according to the formula: $N = Z^2_{1-\alpha/2}\ P(1 - P)/d^2$, where Z score (95% confidence interval; $Z = 1.96$), P is the prevalence of high ORP in the infertile patient ($P = 88.1\%$),[18] and d is the confidence limit around the point estimate ($d = 0.05$). The expected sample size was 160 cases.

Semen analysis

Sperm samples were obtained via masturbation after 3 to 5 days of ejaculatory abstention. Samples were collected into sterile, wide-mouthed containers and liquefied at 37°C. The sperm parameters (liquefaction duration, pH, volume, total sperm count, motility, concentration, morphology) were then evaluated according to the WHO 2010 recommendations.[4] Sperm vitality was determined by staining with eosin Y, and sperm morphology was assessed by evaluating the size and shape of the sperm head and features of the midsection and tail.

Sperm chromatin dispersion test

SDF was determined using the Halosperm® HT-HS10 system (HalotechDNA, Madrid, Spain).[19] A 20-μL semen sample was added to 40 μL of melting agarose, mixed gently using a micropipette, and 8 μL of the cell

suspension was then placed on the treated side of a microscope slide. Slides were stored at 4°C for 10 minutes to solidify the agarose. The slides were then immersed in denaturant agent solution (80 µL of acid denaturation solution to 10 mL of distilled water) and incubated for 7 minutes. The slides were then incubated for 25 minutes in another tray containing 10 mL of tempered lysis solution, the lysis solution was removed by rinsing in distilled water for 5 minutes, and placed in a tray containing 70% ethanol (2 minutes) and 100% ethanol (2 minutes). The sperm were stained with Giemsa stain after drying.[19]

SDF was observed and analyzed by phase-contrast microscopy. A total of 500 sperm were categorized according to Fernandez et al.'s criteria[20]: sperm with DNA fragmentation produce a small halo (halo width $\leq$ one third of the diameter of the core) or no halo, or are degraded (no halo and irregular or poorly stained core); sperm without DNA fragmentation produce a large halo (halo width $\geq$ diameter of the core) or a medium halo (halo size intermediate between big and small halos). The halo classification type is shown in Figure 1. The total score for each halo type was then calculated. The DFI was computed as the proportion of spermatozoa containing fragmented DNA relative to the total number of sperm cells evaluated. A DFI score $<30\%$ was regarded as normal, while a DFI score $\geq 30\%$ was considered abnormal.[20]

ORP

The ORP of the semen samples was measured using the MiOXSYS system (Male Infertility Oxidative System, Caerus, Vilnius, Lithuania). Measurements were generated automatically by pre-insertion of the sensor into the MiOXSYS analyzer followed by loading 30 μL of liquefied semen. ORP readings (mV) were generated after a brief delay. The ORP value was normalized to the concentration of sperm in the semen and given as $mV/10^6$ sperm/mL.[21] Using a clinical cutoff value of $1.34\,mV/10^6$ sperm/mL, individuals were classified into normal ($\leq 1.34\,mV/10^6$ spermatozoa/mL) and abnormal ($>1.34\,mV/10^6$ sperm/mL) ORP groups.[21] This threshold was based

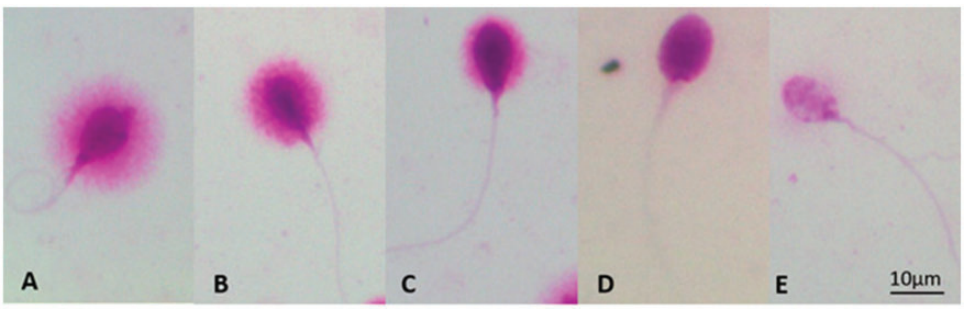

Figure 1. Images of sperm chromatin dispersion test assessed according to halo type (Giemsa stain). Normal group includes spermatozoa with a (a) big or (b) medium halo; abnormal group includes spermatozoa with a (c) small or (d) absent halo, or (e) degraded spermatozoa. A big halo (a) includes halo thickness $\geq$ the length of the minor diameter of the core; a medium halo (b) includes halo thickness $<$ the length of the minor diameter of the core but $>$ one third of the minor diameter of the core; a small halo (c) includes halo thickness $\leq$ one third of the diameter of the minor diameter of the core. An absent halo (d) indicates no halo. Degraded sperm (e) include sperm with no halo and an irregularly or weakly stained core, indicating a subpopulation of spermatozoa with extensive DNA and nuclear protein damage.

on a previous multicenter study by Argawal et al. in 2019, including a large sample size of over 2000 patients.[21]

Statistical analysis

The characteristics of the study population were analyzed using descriptive statistics. Participants were divided into two study groups, with an ORP $\leq 1.34\,mV/10^6$ sperm/mL and an ORP $>1.34\,mV/10^6$ sperm/mL, respectively. The normal distribution of the research variables was determined using the Kolmogorov–Smirnov test. Independent sample *t*-tests (normally distributed variable) or the Mann–Whitney tests (non-normally distributed variable) were employed to examine differences between variables, using independent samples, and relationships between two research variables were determined using Pearson's correlation test. Continuous variables were presented as mean ± standard deviation. Binary data were analyzed using Pearson's χ^2 test. Receiver operating characteristic (ROC) curves was analyzed to determine the diagnostic threshold of ORP for distinguishing between the normal and abnormal semen groups. Differences were considered significant if the P-value was <0.05. All analyses were carried out using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 166 consecutive men from couples diagnosed with infertility were recruited. All participants were involved in every step of the investigation, and no patients were lost from the study during data collection. The baseline characteristics of the 166 men from infertile couples are shown in Table 1. There were no significant differences in age, type of infertility, history of abortion, or duration of abstinence between the normal and abnormal ORP groups.

The sperm concentration ($P<0.001$), total sperm count ($P=0.002$), sperm motility ($P<0.001$), and normal sperm morphology ($P<0.001$) were higher in the normal ORP group than in the abnormal ORP group (Table 2). However, there was no significant difference in the sperm volume or sperm vitality between the groups. The DFI and ORP were significantly higher in men in the abnormal ORP group than in the normal ORP group ($P=0.025$ and $P<0.001$, respectively).

The proportions of patients with normal semen characteristics, such as the sperm concentration, total sperm count, and normal morphology, were significantly higher in the normal ORP group than in the abnormal ORP group (all $P<0.001$) (Table 3). Other sperm characteristics (abnormal head, abnormal neck–tail) were significantly lower in the normal ORP group than in the abnormal ORP group ($P<0.001$), while men with OS had a significantly higher DFI than men without OS ($P=0.013$) (Table 3).

Regarding semen characteristics, ORP was significantly negatively correlated with the sperm concentration ($r=-0.682$, $P<0.001$), total sperm count ($r=-0.485$, $P<0.001$), motility rate ($r=-0.325$, $P<0.001$), and normal morphology ($r=0.493$, $P<0.001$), and significantly positively correlated with the incidence of an abnormal head and neck–tail ($r=0.396$, $P<0.001$; $r=0.324$, $P<0.001$, respectively) (Table 4). In addition, there was a significant positive association between the DFI and OS level ($r=0.238$, $P=0.002$).

The ORP value that predicted sperm parameters was determined by ROC curve analysis (Figure 2). ORP could differentiate between normal and abnormal sperm parameters, with an area under the curve of 0.713% ($P<0.001$). The optimal ORP threshold value for determining sperm

Table 1. Baseline characteristics of men in infertile couples categorized by oxidation–reduction potential.

Characteristic	ORP ≤1.34 (n = 126)	ORP >1.34 (n = 40)	P value
Age (years)	34.35 ± 5.85	34.48 ± 4.84	0.639
<35	73 (57.9)	24 (60.0)	0.856
≥35	53 (42.1)	16 (40.0)	
Infertility type			
Primary	75 (59.5)	21 (52.5)	0.466
Secondary	51 (40.5)	19 (47.5)	
History of abortion			
Yes	33 (26.2)	11 (27.5)	0.840
No	93 (73.8)	29 (72.5)	
Time of abstinence (days)	4.38 ± 1.29	3.88 ± 0.94	0.236

ORP, oxidation–reduction potential (mV/10^6 sperm/mL).
Data are shown as the mean ± standard deviation or number (%).

Table 2. Correlations between semen parameters and sperm analysis.

Semen characteristic	Semen analysis Normal (n = 31)	Abnormal (n = 135)	P value*
Volume (mL)	3.40 ± 1.63	3.13 ± 1.34	0.335
Concentration (×10^6/mL)	47.97 ± 16.07	34.50 ± 17.48	<0.001
Total sperm count (×10^6)	165.70 ± 107.61	111.12 ± 81.99	0.002
Motility (%)	35.42 ± 4.15	23.91 ± 6.94	<0.001
Vitality (%)	85.94 ± 4.07	84.50 ± 5.39	0.166
Normal morphology (%)	4.42 ± 0.72	2.71 ± 1.18	<0.001
Sperm DNA fragmentation			
Big halo (%)	38.83 ± 24.60	33.46 ± 19.98	0.334
Medium halo (%)	45.30 ± 20.74	46.77 ± 15.87	0.952
Small halo (%)	9.35 ± 5.59	11.45 ± 6.63	0.057
No halo (%)	4.30 ± 2.77	5.08 ± 3.80	0.476
Degraded sperm (%)	2.22 ± 1.88	3.25 ± 2.70	0.012
DFI (%)	15.79 ± 8.38	19.78 ± 10.02	0.025
ORP (mV/10^6 sperm/mL)	0.53 ± 0.19	1.04 ± 0.93	<0.001

DFI, DNA fragmentation index; ORP, oxidation–reduction potential.
Data are shown as the mean ± standard deviation or number (%).
*Compared using independent sample *t*-test (normally distributed variables) or Mann–Whitney test (non-normally distributed variables).

quality was 0.77 mV/10^6 sperm/mL, with a sensitivity of 50.4%, specificity of 93.5%, positive predictive value of 52.9%, and negative predictive value of 32.3%.

Discussion

OS has been reported to be a primary cause of male infertility, resulting in aberrant sperm parameters and elevated SDF levels.[22,23] Agarwal et al. showed that 25% of infertile men had elevated levels of ROS in their sperm,[24] consistent with our current findings. OS has been shown to adversely affect fertility, embryo development, and pregnancy rates.[13,25] In terms of disorders caused by OS, Barati et al. concluded that OS in the testes and sperm was

Table 3. Associations between oxidative stress and semen characteristics.

Semen parameter	ORP $\leq$1.34 (n = 126)	ORP >1.34 (n = 40)	P value*
Volume (mL)	3.24 ± 1.48	3.00 ± 1.11	0.348
1.5	5 (4.0)	2 (5.0)	0.675
≥1.5	121 (96.0)	38 (95.0)	
Concentration ($\times 10^6$/mL)	43.35 ± 15.18	17.08 ± 9.43	<0.001
<15	1 (0.8)	14 (35.0)	<0.001
≥15	125 (99.2)	26 (65.0)	
Total sperm count ($\times 10^6$)	143.3 ± 90.51	51.94 ± 34.19	<0.001
Motility (%)	28.24 ± 6.78	19.20 ± 7.29	<0.001
<32	84 (66.7)	39 (97.5)	<0.001
≥32	42 (33.3)	1 (2.5)	
Vitality (%)	84.90 ± 5.50	84.38 ± 4.07	0.581
<58	1 (0.8)	0 (0)	0.759
≥58	125 (99.2)	40 (100)	
Normal morphology (%)	3.40 ± 1.13	1.85 ± 1.05	<0.001
<4	63 (50)	38 (95)	<0.001
≥4	63 (50)	2 (5)	
Abnormal head (%)	94.16 ± 2.24	96.23 ± 2.57	<0.001
Abnormal neck–tail (%)	46.31 ± 6.18	51.90 ± 8.59	<0.001
Sperm DNA fragmentation			
Big halo (%)	35.15 ± 21.04	32.30 ± 20.77	0.402
Medium halo (%)	46.83 ± 7.43	45.44 ± 14.91	0.517
Small halo (%)	10.27 ± 5.42	13.55 ± 8.70	0.037
No halo (%)	4.73 ± 3.44	5.57 ± 4.17	0.338
Degraded sperm (%)	3.03 ± 2.59	3.15 ± 2.65	0.834
DFI (%)	17.98 ± 8.98	22.37 ± 1.67	0.013

DFI, DNA fragmentation index; ORP, oxidation–reduction potential (mV/10^6 sperm/mL).
*Data are shown as the mean ± standard deviation or number (%).
*Compared using the independent sample *t*-test (normally distributed variables) or Mann–Whitney test (non-normally distributed variables), and the χ^2 test.

Table 4. Correlations between semen parameters and oxidation–reduction potential for all participants.

Factor	ORP (mV/10^6 sperm/mL) r	P value*
Volume (mL)	−0.093	0.233
Concentration ($\times 10^6$/mL)	−0.682	<0.001
Total sperm count ($\times 10^6$)	−0.485	<0.001
Motility (%)	−0.325	<0.001
Vitality (%)	−0.006	0.935
Normal morphology (%)	−0.493	<0.001
Abnormal head (%)	0.396	<0.001
Abnormal neck and tail (%)	0.324	<0.001
DFI (%)	0.238	0.002

DFI, DNA fragmentation index; ORP, oxidation–reduction potential.
*Pearson's correlation test.

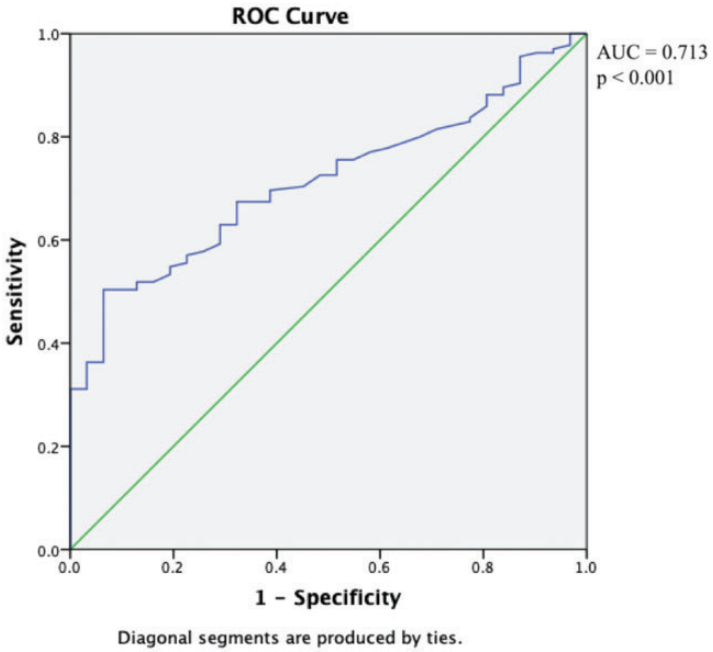

Figure 2. Receiver operating characteristic (ROC) curves were used to identify the optimal oxidation–reduction potential for predicting semen parameters.
AUC: area under the curve.

negatively associated with sperm motility and acrosome activity in obese individuals,[26] while obesity enhanced the metabolic rate and generation of ROS in testicular tissue and sperm, and increased the risk of infertility by 20%. Maintaining a healthier lifestyle and engaging in regular exercise to maintain a healthy weight and body mass index can thus improve sperm quality and minimize the incidence of sperm abnormalities.[27]

Comparing the levels of OS markers in normal men with and without varicocele revealed that individuals with varicocele had a considerably increased risk of OS.[28] Infertile patients with varicocele also showed elevated ROS levels in their semen, including ROS, nitric oxide, and lipid peroxidation products.[29] Under these conditions, excess ROS could damage sperm cell membranes and chromatin, resulting in lipid peroxidation and poor sperm quality.[30] In a study of smokers,[31] elevated levels of cadmium and lead in the blood and sperm increased ROS and affected sperm motility. In addition, testicular cancer has been linked to OS in the testicles, which was shown to affect sperm parameters.[32] Overall, the above results revealed negative impacts of OS on sperm quality, in either molecular terms or in semen analysis results. The outcomes of the current investigation were largely consistent with these previous studies. Notably, the present results revealed a correlation between ORP and semen analysis, although other potentially pertinent criteria, such as medical condition, anthropometry, and medical history, were not considered.

The present study also identified the effect of OS on the integrity of sperm DNA. An oxidation–reduction imbalance

may lead to an increase in SDF.[33] The oxidative radicals 8-hydroxy-2-deoxyguanosine and two ethenonucleosides (1, N6-ethenoadenosine and 1, N6-ethenoguanosine) have been identified as indicators of SDF. The growth of periscrotal adipose tissue in obese men increases the scrotal temperature and ROS generation, leading to decreased stability of sperm DNA and an increased DFI. Patients with diabetes and obesity had significantly mean ($\pm$ standard deviation) higher levels of ROS and SDF than the control group (ROS: 66.03 ± 6.77 vs. 40.85 ± 0.74 relative light units [RLU]/s/x10^6 sperm; SDF%: $49.12\% \pm 3.18\%$ vs. $26.38\% \pm 1.63\%$, $P<0.05$).[34] These results suggest that elevated levels of ROS and DFI could be used as fertility predictors in men with diabetes or obesity. An *in vitro* investigation linked mitochondrial malfunction to sperm immobility, involving the inactivation of genes governing electron transport proteins, particularly ATP synthesis.[35] Moreover, ROS may damage mitochondrial DNA, resulting in impaired sperm motility caused by OS. Men with leukocytospermia showed higher levels of ROS and DFI than the control group (ROS: 1839.65 ± 2173.57 RLU/s vs. 1101.09 ± 5557.54 RLU/s, $P = 0.002$; DNA damage: $26.47\% \pm 19.64\%$ vs. $19.89\% \pm 17.31\%$, $P = 0.047$).[36] In addition, SDF was associated with apoptosis, a reduced fertilization rate, and an increased risk of miscarriage.[37] A study published in 2022 showed that increased seminal ORP had a negative impact on the fertilization process, blastocyst development, implantation/clinical pregnancy, and the live-birth rate.[38] Although we did not examine the outcomes of treatment cycles in infertile individuals, the deleterious effect of OS, measured by ORP assay, on sperm DNA stability has been extensively reported.[39] In cases of high ORP, it is important to monitor the progressive impact on male reproductive function and pregnancy outcomes, to pave the path for further investigations focusing on managing OS, measured by ORP, in efforts to improve infertility treatment outcomes.

ROS can be evaluated by chemiluminescence test, lipids by thiobarbituric acid assay or 4-hydroxynonenal, antioxidants by colorimetric assay, and apoptotic markers, such as annexin V, by colorimetric assay[40]; however, these procedures are expensive and time-consuming, and may require sophisticated apparatus. Most ROS assays use one or more probes with variable sensitivity and specificity, and the use of different probes with different sensitivities and specificities will produce distinct outcomes. Notably, early assessment of OS levels might help to define the diagnosis and treatment solutions for male infertility. The MiOXSYS system is a revolutionary system for quantifying ORP in human sperm.[7,21] The method is inexpensive, accurate, and user-friendly, requiring neither capital equipment expenditure nor highly qualified employees.[40] ORP proved to be a useful measure integrating measurements of oxidants and reductants, which can be used for analyzing abnormal and normal semen in patients. The MiOXSYS system is a precise, simple technique that can rapidly analyze seminal OS in small volumes of semen, including cryopreserved samples. ORP measurement is also a low-cost method compared with chemiluminescence and flow cytometry, which both require expensive, computerized equipment. ORP measurement is thus widely applicable in clinical and research settings. Notably, Agarwal et al. advised that ORP could be used as an independent method to assess sperm quality in infertile patients.[7,40] To the best of our knowledge, the current study is the first in Vietnam to use this advanced technique to examine the degree of ROS in semen samples. Our findings will thus contribute to the literature regarding the negative effects of oxidant radicals

on sperm quality, particularly in Asian populations.

This study has some limitations. In addition to the small sample size, we did not examine several variables that might directly influence ORP outcomes and sperm quality, which might act as confounding variables in the investigation of the effect of ORP on sperm analysis outcomes. In addition, we only measured OS using ORP, and did not compare the diagnostic efficacy of ORP with other analytical approaches (e.g., total antioxidant capacity, enzyme measurement). Therefore, further studies with larger sample sizes and more thorough method designs are required to confirm our results.

In conclusion, measurement of the ORP demonstrated that OS might have an adverse impact on sperm quality, in terms of sperm motility, concentration, morphology, vitality, and DFI. The optimal ORP threshold for determining sperm quality is $0.77\,mV/10^6$ sperm/mL. This approach should be used as a screening test for assessing male reproductive capacity in infertile couples.

Author contributions

LMT, NDN, and NVT developed the study concept and designed the study; LMT, NDN, and DTHN acquired the data for analysis; NDN performed the statistical analysis; NDN and LMT drafted the first manuscript; all authors contributed to the interpretation of the data and provided critical revision for important intellectual content and approved the final manuscript.

Data availability statement

The dataset used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

Declaration of conflicting interests

The author(s) declares that there is no conflict of interest.

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References

1. Ghuman N and Ramalingam M. Male infertility. *Obstet Gynaecol Reprod Med* 2018; 28: 7–14.
2. Sun H, Gong TT, Jiang YT, et al. Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990-2017: results from a global burden of disease study. *Aging (Albany NY)* 2019; 11: 10952–10991.
3. Naz M and Kamal M. Classification, causes, diagnosis and treatment of male infertility: a review. *Orient Pharm Exp Med* 2017; 17: 89–109.
4. WHO. WHO laboratory manual for the examination and processing of human semen, Sixth edition. World Health Organization; 2021.
5. WHO. WHO laboratory manual for the examination and processing of human semen, Fifth edition. World Health Organization; 2010.
6. Lanzafame FM, Vignera SL, Vicari E, et al. Oxidative stress and medical antioxidant treatment in male infertility. *Reprod Biol* 2009; 19: 638–659.
7. Agarwal A, Roychoudhury S, Bjugstad KB, et al. Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? *Ther Adv Urol* 2016; 8: 302–318.
8. Homa S, Vassiliou A, Stone J, et al. A comparison between two assays for measuring seminal oxidative stress and their relationship with sperm DNA fragmentation and semen parameters. *Genes (Basel)* 2019; 10: 236.

9. Gyun JS, Edward PN and Vivian L. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. *Int J Androl* 2006; 29: 569–575.
10. Fraczek M, Lewandowska A, Budzinska M, et al. The role of seminal oxidative stress scavenging system in the pathogenesis of sperm DNA damage in men exposed and not exposed to genital heat stress. *Int J Environ Res Public Health* 2022; 19: 2713.
11. Hitit M, Ugur MR, Dinh TTN, et al. Cellular and functional physiopathology of bull sperm with altered sperm freezability. *Front Vet Sci* 2020; 23: 581137.
12. Ugur MR, Saber Abdelrahman A, Evans HC, et al. Advances in cryopreservation of bull sperm. *Front Vet Sci* 2019; 27: 268.
13. Agarwal A, Sharma RK, Nallella KP, et al. Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril* 2006; 86: 878–885.
14. Valko M, Izakovic M, Mazur M, et al. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem* 2004; 266: 37–56.
15. Agarwal A, Parekh N, Panner Selvam MK, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *World J Mens Health* 2019; 37: 296–312.
16. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573–577.
17. WHO World Health Organization. *International Classification of Diseases, 11th Revision (ICD-11)* Geneva: WHO 2018.
18. Gill K, Kups M, Harasny P, et al. The negative impact of varicocele on basic semen parameters, sperm nuclear DNA dispersion and oxidation-reduction potential in semen. *Int J Environ Res Public Health* 2021; 18: 5977.
19. Le MT, Nguyen TAT, Nguyen HTT, et al. Does sperm DNA fragmentation correlate with semen parameters? *Reprod Med Biol* 2019; 18: 390–396.
20. Fernández JL, Muriel L, Goyanes V, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. *Fertil Steril* 2005; 84: 833–842.
21. Agarwal A, Selvam MKP, Arafa M, et al. Multi-center evaluation of oxidation-reduction potential by the MiOXSYS in males with abnormal semen. *Asian J Androl* 2019; 21: 565–569.
22. Evenson D and Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. *Reprod Biomed Online* 2006; 12: 466–472.
23. Aitken RJ, Drevet JR, Moazamian A, et al. Male infertility and oxidative stress: a focus on the underlying mechanisms. *Antioxidants (Basel)* 2022; 11: 306.
24. Agarwal A, Saleh RA and Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003; 79: 829–843.
25. Tremellen K. Oxidative stress and male infertility-a clinical perspective. *Hum Reprod Update* 2008; 14: 243–258.
26. Barati E, Nikzad H and Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell Mol Life Sci* 2020; 77: 93–113.
27. Leisegang K and Dutta S. Do lifestyle practices impede male fertility? *Andrologia* 2021; 53: e13595.
28. Blumer CG, Restelli AE, Giudice PT, et al. Effect of varicocele on sperm function and semen oxidative stress. *BJU Int* 2012; 109: 259–265.
29. Mehraban D, Ansari M, Keyhan H, et al. Comparison of nitric oxide concentration in seminal fluid between infertile patients with and without varicocele and normal fertile men. *Urol J* 2005; 2: 106–110.
30. Cho CL, Esteves SC and Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. *Asian J Androl* 2016; 18: 186–193.
31. Kiziler AR, Aydemir B, Onaran I, et al. High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage

in infertile subjects. *Biol Trace Elem Res* 2007; 120: 82–91.
32. Ritchie C and Ko EY. Oxidative stress in the pathophysiology of male infertility. *Andrologia* 2021; 53: e13581.
33. González-Marín C, Gosálvez J and Roy R. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. *Int J Mol Sci* 2012; 13: 14026–14052.
34. Abbasihormozi SH, Babapour V, Kouhkan A, et al. Stress hormone and oxidative stress biomarkers link obesity and diabetes with reduced fertility potential. *Cell J* 2019; 21: 307–313.
35. Nowicka-Bauer K, Lepczynski A, Ozgo M, et al. Sperm mitochondrial dysfunction and oxidative stress as possible reasons for isolated asthenozoospermia. *J Physiol Pharmacol* 2018; 69: 403–417.
36. Agarwal A, Mulgund A, Alshahrani S, et al. Reactive oxygen species and sperm DNA damage in infertile men presenting with low-level leukocytospermia. *Reprod Biol Endocrinol* 2014; 12: 126.
37. Alvarez Sedo C, Bilinski M, Lorenzi D, et al. Effect of sperm DNA fragmentation on embryo development: clinical and biological aspects. *JBRA Assist Reprod* 2017; 21: 343–350.
38. Henkel R, Morris A, Vogiatzi P, et al. Predictive value of seminal oxidation-reduction potential analysis for reproductive outcomes of ICSI. *Reprod Biomed Online* 2022; 45: 1007–1020.
39. Panner Selvam MK, Baskaran S, O'Connell S, et al. Association between Seminal Oxidation-Reduction potential and sperm DNA fragmentation-a meta-analysis. *Antioxidants (Basel)* 2022; 11: 1563. doi: 10.3390/antiox11081563.
40. Agarwal A and Majzoub A. Laboratory tests for oxidative stress. *Indian J Urol* 2017; 33: 199–206.