Oxidation-reduction potential as a new marker for oxidative stress: Correlation to male infertility

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Male infertility affects men worldwide. Oxidative stress (OS), characterized by an overabundance of reactive oxygen species (ROS) or a deficiency of antioxidants, is one of the major causes of male infertility. OS causes damage at the molecular level, which impairs lipids, proteins, and DNA. The cyclic cascade of redox reactions weakens sperm function which leads to poor semen parameters and eventual sterility. There is a need for advanced diagnostic tests that can quickly and accurately detect OS. Most commonly used assays can only measure single constituents of OS. However, the MiOXSYS System introduces a new strategy to detect OS by measuring the oxidation-reduction potential (ORP)--a direct evaluation of the redox balance between ROS and antioxidants. The MiOXSYS System has shown promise as a diagnostic tool in the evaluation of male infertility. This review explores the concept of ORP, details the principle of the MiOXSYS System, and summarizes the findings in clinical studies that support ORP measurement in semen.

Keywords: Diagnosis; Male infertility; Oxidative stress; Reactive oxygen species; Spermatozoa

INTRODUCTION

Approximately 7% of men worldwide are affected by male infertility, of which male factors contribute to 40%–50% of all infertility cases [1]. The basic semen analysis remains as the standard of care to initially evaluate male infertility despite its controversies in clinical effectiveness [2,3]. The test yields highly variable results from the same individual, inconsistent semen parameters among different observers, and no information about sperm dysfunctions at the molecular level. Additionally, the 2010 World Health Organization's reference values might not be applicable to all men as their inclusion criteria lacks diverse patient populations [4]. Therefore, more advanced tests of sperm function may assist in accurately diagnosing male infertility.

Oxidative stress (OS) is highly implicated in the pathogenesis of male infertility [5-8]. OS occurs when production of reactive oxygen species (ROS) outweighs the concentration of antioxidants in human semen. Physiologically, ROS are vital for sperm maturation as they undergo capacitation, hyperactivation, acrosome reaction, and oocyte fusion [9,10]. However, excess ROS results in lipid peroxidation, DNA damage, and induction of apoptosis, all of which trigger a vicious cycle of OS [11]. Clinically, OS can translate into reduced fertilization rates, failure of implantation, impaired embryonic development, recurrent

pregnancy loss and poor assisted reproductive technology (ART) outcomes [12-17].

Early and accurate detection of OS ensures a better prognosis for infertile men. Currently available assays include chemiluminescence for ROS, total antioxidant capacity (TAC) for antioxidants, and malondialdehyde (MDA) assay for *post hoc* damage from lipid peroxidation. However, these tests carry certain disadvantages such as high cost, sophisticated instrumentation, large sample volumes, complex methodologies, and extensive technical training [18-22] (Supplementary Table 1). Most importantly, they only capture a single dimension of OS, quantifying either ROS or antioxidants. Thus, a test that includes all of the constituents of OS may provide a better understanding of the true redox state and facilitate better management.

Measuring oxidation-reduction potential (ORP) is the latest advancement in male infertility diagnostics. ORP, also known as the redox balance, is a direct measure of OS as it describes the relative proportions of oxidants (ROS) to reductants (antioxidants). Previous clinical studies have been successful in evaluating ORP in the blood of patients with traumatic brain injury, stroke, metabolic syndrome, liver toxicity, sepsis, and exercise-induced OS [23-31]. ORP measurement has recently been tested in human semen by a number of andrology laboratories to determine whether it is a reliable indicator of OS. The objective of this review is to (1) describe the causes and mechanisms of OS-induced damage in male infertility; (2) briefly discuss the mechanics of the MiOXSYS System, a device that measures ORP; (3) summarize the data produced by recent clinical studies; and (4) introduce studies in progress that may pave the way for utilization of ORP in clinical practice.

CAUSES AND MECHANISMS OF OXIDATIVE STRESS-INDUCED DAMAGE IN MALE INFERTILITY

There are multiple endogenous and exogenous factors responsible for poor sperm quality in an infertile man. They cause infertility by producing a surplus of ROS targeted towards healthy spermatozoa. Examples of conditions that elevate ROS include: genital tract infections, varicocele, spinal cord injury, diabetes, obesity, tobacco smoking, alcohol use, recreational drug abuse, ionizing radiation, psychological stress, strenuous exercise, or air pollutants [32-34] (Fig. 1). Two major sources of ROS are leukocytes via the hexose monophosphate shunt and immature spermatozoa at the level of the plasma membrane or mitochondria [35].

Spermatozoa are highly susceptible to ROS due to the specific composition of their plasma membrane. They contain polyunsaturated fatty acids, which are structurally unstable and highly prone to lipid peroxidation [36]. Electrophilic aldehyde byproducts, such as MDA, 4-hydroxynonenal, and acrolein, are generated from the peroxidative damage, which
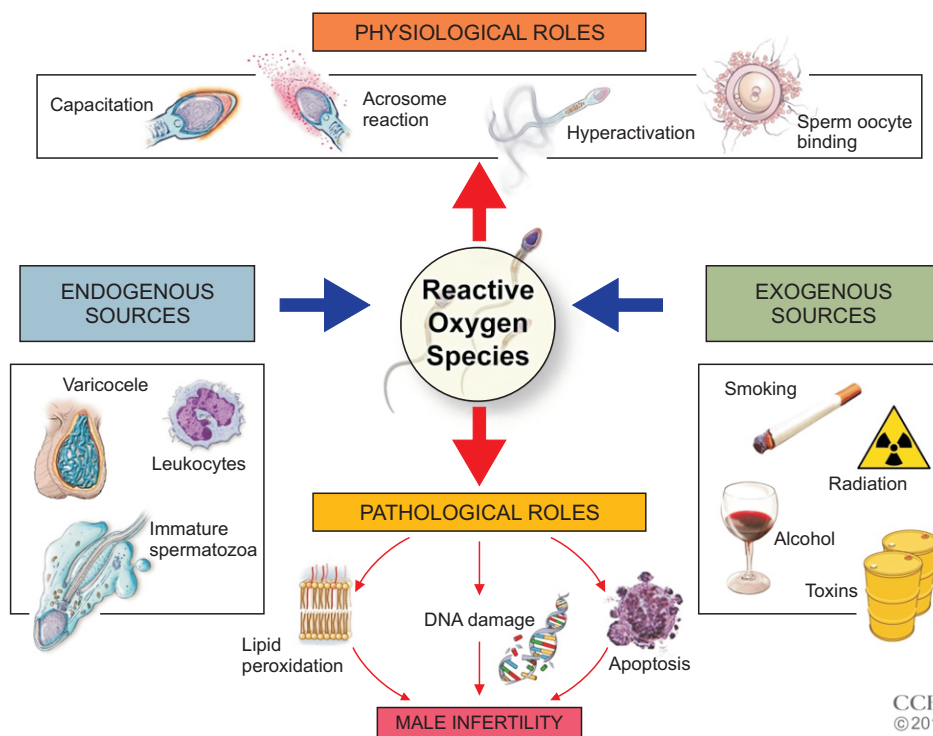

Fig. 1. Schematic of the physiological/pathological roles of reactive oxygen species and sources leading to increased production. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

negatively affects DNA integrity, mitochondrial function, apoptosis, and cellular signaling [8,37,38]. As aforementioned, the mitochondria are not only a target of ROS, but they are also a major source [39]. Thus, prolonged damage to the inner mitochondrial membrane will create a continuous cycle of ROS production that induces sterility. Overall, there is strong clinical evidence suggesting that OS is linked with male infertility as shown through various meta-analyses of infertile patients with OS risk factors (i.e., varicocele, chronic prostatitis, mobile phone use, cigarette smoking) [40-44].

MEASUREMENT OF OXIDATION-REDUCTION POTENTIAL: PRINCIPLE OF THE MiOXSYS SYSTEM AND OTHER RELEVANT DEVICES

The MiOXSYS System is a novel technology designed to measure the transfer of electrons from reductants to oxidants in human semen samples. ORP is calculated with the Nernst equation: ORP=E^o-RT/nF ln([Red]/[Ox]) where E^o=standard reduction potential, R=universal gas constant, T=absolute temperature, n=number of moles of exchanged electrons, F=Faraday's constant, [Red]=concentration of reduced species, [Ox]=concentration of oxidized species [45]. To start the procedure, disposable test sensors—each equipped with three electrodes—are inserted into a galvanostatic analyzer (Fig. 2). Once the sample is applied onto the sample port, it will eventually reach the reference electrode, complete the electrochemical circuit, and signal the analyzer to emit a low voltage oxidizing current between electrodes. This current assists in generating an ORP from which the average is calculated from the final 10 seconds of the trial run [46]. The ORP value appears on the analyzer display screen as millivolts (mV), which parallels the degree

of OS. A higher ORP corresponds to higher levels of oxidant activity [47].

Similar ORP devices exist in the market but are used to monitor water sanitation, metal finishing, and ozone treatment [48-52]. To the best of our knowledge, the MiOXSYS System is the first ORP device to test human semen samples. It is specifically calibrated to detect human sperm at 0.1–400 mV. Requiring only 30 µL of sample volume and less than 5 minutes for the entire procedure, the MiOXSYS System yields an ORP value with a high sensitivity, specificity, and accuracy [53-58]. Fresh or frozen semen and seminal plasma can be also be evaluated [59]. From a technical perspective, the MiOXSYS System is easy to use, inexpensive, less time consuming, and more efficient in providing reliable results. Factors that affect ORP measurement include advanced semen age, poor semen liquefaction, or repeated centrifugation [60-63].

CLINICAL SIGNIFICANCE OF OXIDATION-REDUCTION POTENTIAL MEASUREMENT

Using the MiOXSYS System, the investigators at Cleveland Clinic's American Center for Reproductive Medicine began measuring ORP to detect OS in human semen samples. Recent clinical studies have generated significant data that enabled ORP measurement to quickly gain momentum as a robust diagnostic tool for male infertility. As a clarification, earlier studies looked at both static ORP (sORP) and antioxidant capacity reserve (cORP) [53]. sORP provides a composite measure of available oxidants and reductants whereas cORP denotes the amount of accessible reductants to combat OS. Due to more promising data, sORP became the topic of interest and was mostly referred to as
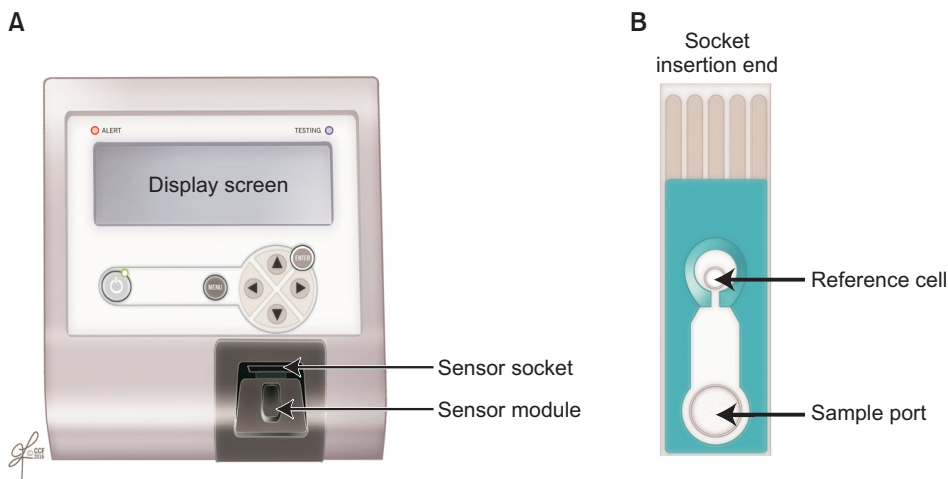

Fig. 2. Setup of the MiOXSYS (A) analyzer and sensor (B).

"ORP" in later studies [54-58]. All studies were conducted with at least a power of 80% with statistical significance set at p<0.05. To calculate ORP (as normalized ORP), the raw ORP value (mV) was divided by the sperm concentration (sperm count × 10^6/mL). Normalized ORP was expressed as mV/10^6 sperm/mL. Additional information on the qualitative value of ORP measurement such as its unique ability in predicting abnormal semen parameters and identifying infertile men are elaborated with details of each clinical study published (Table 1).

1. ORP in human semen and seminal plasma

Agarwal et al. [53] initiated a pilot study to measure ORP in human semen and seminal plasma. Another goal of the study was to observe the relationship between ORP and semen parameters across time. The investigators recruited a small cohort of 26 healthy controls and 33 infertile men. Semen samples were analyzed with a basic semen analysis per the 2010 World Health Organization guidelines and ORP measurement at 0 and 120 minutes after semen liquefaction. Compared to those of the controls, semen samples from infertile patients exhibited reduced motility and morphology (p<0.01). Additionally, they had elevated ORP levels in the seminal plasma at 120 minutes (p=0.036). Control and infertile patients were combined and categorized based on normal and abnormal motility (<40%). Individuals with poor sperm motility had lower concentrations, total count, and morphology (p<0.05). Their ORP levels were elevated in semen and seminal plasma at 120 minutes (p=0.035 and p=0.04, respectively). ORP was related to abnormal semen parameters as there was a significant inverse relationship of ORP with concentration and total sperm count at 0 and 120 minutes (p<0.05) (Supplementary Figs. 1-4). Via a receiver operating characteristic (ROC) curve analysis, an ORP cutoff value of 1.48 mV/10^6 sperm/mL in semen and 2.09 mV/10^6 sperm/mL in seminal plasma was proposed to aid in identifying abnormal semen samples, specifically those with <40% motility (Supplementary Fig. 5). Distributions of

Table 1. List of key findings on ORP and male infertility in clinical studies

Study	Key findings
Agarwal et al. [53] (2016)	1. ORP can be measured in semen and seminal plasma 2. ORP measurements are stable up to 120 minutes 3. Negative correlation between ORP and semen concentration/total sperm count regardless of fertility status
Agarwal et al. [54] (2017)	1. Elevated levels of ORP in infertile patients compared to those of controls 2. Negative correlation between ORP and semen parameters (concentration, total sperm count, motility, and morphology) in all subjects 3. ORP value of 1.36 mV/10^6 sperm/mL to distinguish fertile from infertile men 4. Low intraobserver and interobserver variability
Agarwal and Wang [55] (2017)	1. Elevated levels of ORP in abnormal semen parameters (of infertile patients) 2. Sperm concentration and motility improves as ORP decreases 3. ORP value of 2.59 mV/10^6 sperm/mL best predicts oligozoospermia 4. A combination of ORP and at least 1 other abnormal semen parameter (especially concentration) is more robust in identifying OS than ORP alone
Agarwal et al. [56] (Forthcoming)	1. ORP is highly predictive of OZ and AZ samples regardless of fertility status. There is a weaker predictive value for TZ and OAT samples 2. ORP is best at predicting OZ samples 3. Elevated levels of ORP in all subgroups when compared to NZ samples 4. A combination of ORP and two other abnormal semen parameters is more robust in identifying OS than ORP alone
Agarwal et al. [57] (2017)	1. Collectively and individually between the Cleveland Clinic and Doha, Qatar, there were poorer semen parameters and higher ORP values present in infertile semen samples when compared to those of healthy controls. 2. No significant differences between infertile groups in both data sets except progressive motility and morphology 3. No significant differences between control groups in both data sets except morphology 4. Elevated levels of ORP in abnormal semen groups 5. ORP value of 1.42 mV/10^6 sperm/mL to distinguish fertile from infertile men
Arafa et al. [58] (2017)	1. ORP value of 1.38 mV/10^6 sperm/ml to distinguish normal from abnormal semen samples 2. ORP value of 1.41 mV/10^6 sperm/mL to distinguish fertile from infertile men

ORP, oxidation-reduction potential; OZ, oligozoospermic; AZ, asthenozoospermic; TZ, teratozoospermic; OAT, oligoasthenoteratozoospermic; NZ, normozoospermic.

ORP values and the median in relation to the cutoffs are also displayed in Supplementary Fig. 5. The proposed ROC values were not statistically robust as it yielded low positive predictive values (PPVs) (45% in semen, 46.7% in seminal plasma). This most likely occurred due to a limited number of men with proven fertility in the control group. Including more individuals in the control group with similar baseline characteristics would increase the effectiveness of the assay. Overall, this study demonstrated that ORP can be detected in both semen and seminal plasma up to 120 minutes with the ability to perhaps distinguish abnormal semen quality.

2. ORP in male infertility, establishment of an ORP reference value, and reliability of assay

In a second study, Agarwal et al. [54] further attempted to validate ORP as a surrogate marker in predicting abnormal semen. In this study, they enrolled a larger sample of 51 healthy controls (15 proven fertility and 36 unproven fertility) and 106 infertile patients (38 with varicocele, 13 idiopathic, 55 nonclassified). At baseline, the infertile patients had lower sperm concentrations, total sperm count, motility, and morphology ($p \leq 0.001$). ORP levels were significantly higher in infertile patients versus controls (6.22 ± 1.10 mV/10^6 sperm/mL and 1.59 ± 0.29 mV/10^6 sperm/mL, p=0.004). However, as a collective group, ORP was negatively correlated with concentration, total sperm count, motility, and morphology (p<0.01). ROC curve analysis yielded a normalized cut-off value of 1.36 mV/10^6 sperm/

mL with a 69.6% sensitivity, 83.1% specificity, 85.3% PPV, 65.9% negative predictive value (NPV), and 75.2% accuracy (area under the curve [AUC]=0.770) (Fig. 3). Median ORP values of the infertile patients were above this cut-off value whereas those of the controls were below (p=0.004) (Fig. 4). The same trend was observed when comparing subcategories of patients and controls (Supplementary Fig. 6). Solely measuring ORP appeared to be effective in both determining excess OS in semen samples and differentiating between infertile patients and controls.

Intraobserver and interobserver reliability was tested by having different observers analyze multiple samples in replicates (Supplementary Fig. 7). The goal was to determine if ORP measurement provided more objective data as opposed to conventional semen analysis where high variability is an issue. This was expressed as a coefficient of variation (%CV). Variability for any one observer and across observers were 8.39% and 3.61%, respectively. Overall, this study supported ORP as a highly reproducible measurement and found that a normalized ORP cutoff of 1.36 mV/10^6 sperm/mL may have discriminative capabilities.

Although ORP values correlated strongly with semen parameters, this study did not further examine whether they were *predictive* of poor semen quality. This was an important question to answer since ORP in addition to abnormal semen parameters would theoretically be a more robust determination of OS status and male infertility.
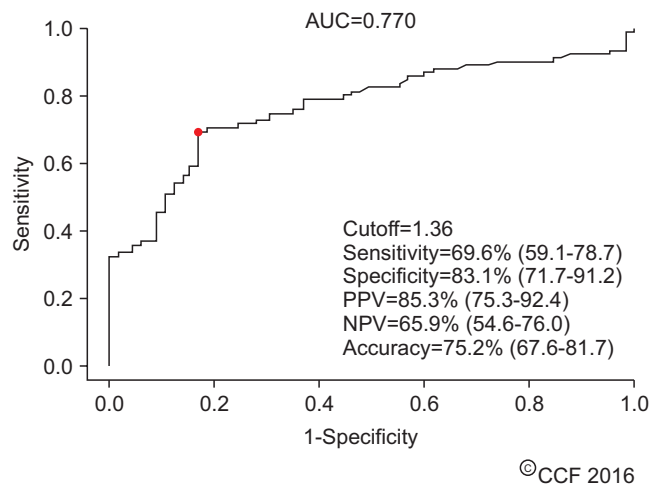

Fig. 3. A receiver operating characteristic curve was used to identify the static oxidation-reduction potential (mV/10^6 sperm/mL) criterion i.e., cutoff, sensitivity, and specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and area under the curve (AUC) that best predicted the normal and abnormal semen parameters as well as differentiated normal healthy controls from male factor infertility patients. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

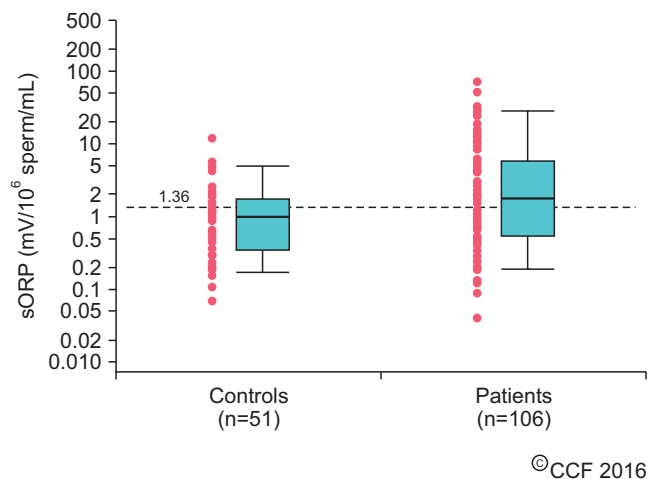

Fig. 4. Distribution of static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) in controls and patients showing the established cutoff values. Data are represented as box-plot showing median and interquartile range. The whiskers are the 95% confidence intervals. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

3. ORP predictive value and behavior over time in infertile patients

Agarwal and Wang [55] sought to determine the predictive value of ORP and its relationship to semen parameters over time. Semen samples from 49 healthy controls and 194 infertile patients were obtained. Similar to previous studies, the infertile patients had abnormal semen parameters and elevated ORP levels compared with those of the controls (p<0.001). Significant negative correlations between ORP and certain semen parameters were also found in the infertile men (p<0.05). Using the 2010 WHO guidelines, semen samples from the infertile patients were arranged into normal and abnormal semen parameters (oligozoospermia, asthenozoospermia, and teratozoospermia). ORP levels were elevated in all abnormal semen parameters, the highest value belonging to the oligozoospermic group (p<0.001). In fact, ORP was the most predictive of oligozoospermic samples at a cutoff of 2.59 mV/10^6 sperm/mL with 88% sensitivity, 91.2% specificity, 90% PPV, 89.4% NPV, and 89.7% accuracy (AUC=0.754) (Fig. 5). This coincides with the negative correlation between ORP and concentration. This is also a

logical observation since ORP is normalized to concentration.

Additional ROC curve analysis demonstrated that an ORP cutoff of 1.57 mV/10^6 sperm/mL achieving 70.4% sensitivity, 88.1% specificity, 95.5% PPV, 45.1% NPV, and 74.2% accuracy (AUC=0.809) can detect at least 1 abnormal semen parameter (p<0.001) (Fig. 6). There was a greater range of ORP values in infertile patients with abnormal sperm versus that of infertile patients with normal sperm. This suggests that ORP fluctuates as a reflection of abnormal semen parameters induced by OS. Nevertheless, the majority of patients with abnormal semen parameters had an ORP well above the cutoff value.

These reference values confirm prior data showing that ORP is related to semen parameters. Interestingly, because ORP best predicts oligozoospermia, it is very likely that OS strongly plays a pathologic role in this subset of patients [7,64]. It is important to not forget that OS contributes to other seminal abnormalities as well. This is reflected through different ORP cutoffs for each abnormal semen parameter. This study reveals that a combined approach of considering ORP and at least 1 abnormal semen parameter
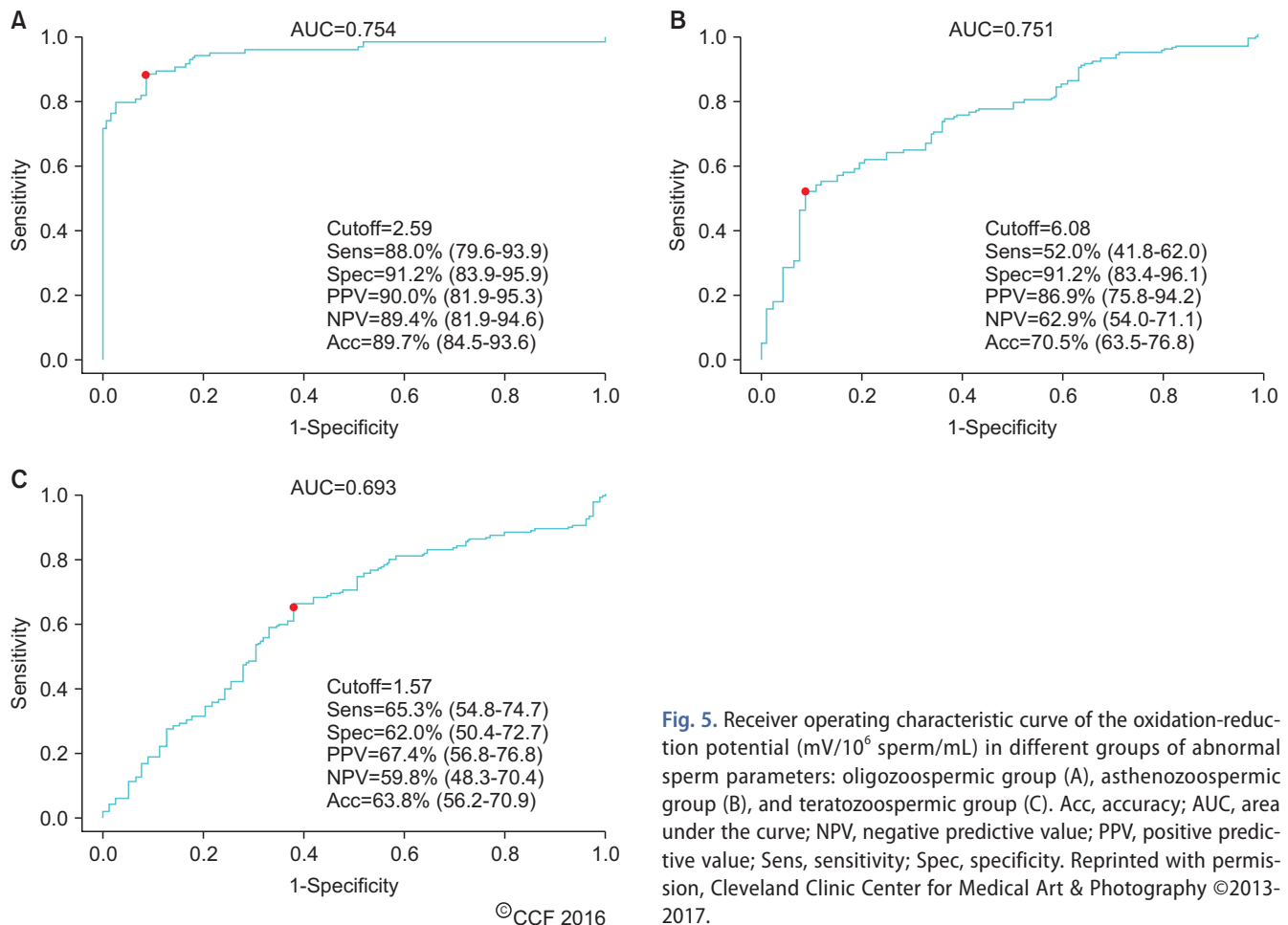

A

AUC=0.754

Cutoff=2.59
Sens=88.0% (79.6-93.9)
Spec=91.2% (83.9-95.9)
PPV=90.0% (81.9-95.3)
NPV=89.4% (81.9-94.6)
Acc=89.7% (84.5-93.6)

B

AUC=0.751

Cutoff=6.08
Sens=52.0% (41.8-62.0)
Spec=91.2% (83.4-96.1)
PPV=86.9% (75.8-94.2)
NPV=62.9% (54.0-71.1)
Acc=70.5% (63.5-76.8)

C

AUC=0.693

Cutoff=1.57
Sens=65.3% (54.8-74.7)
Spec=62.0% (50.4-72.7)
PPV=67.4% (56.8-76.8)
NPV=59.8% (48.3-70.4)
Acc=63.8% (56.2-70.9)

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Fig. 5. Receiver operating characteristic curve of the oxidation-reduction potential (mV/10^6 sperm/mL) in different groups of abnormal sperm parameters: oligozoospermic group (A), asthenozoospermic group (B), and teratozoospermic group (C). Acc, accuracy; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

Fig. 6. (A) Receiver operating characteristic curve of the oxidation-reduction potential (ORP) (mV/10^6 sperm/mL) in detecting at least 1 sperm parameter. (B) Box-and-whisker plots showing the distributions of the ORP cutoff between normal and abnormal sperm parameters. Acc, accuracy; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
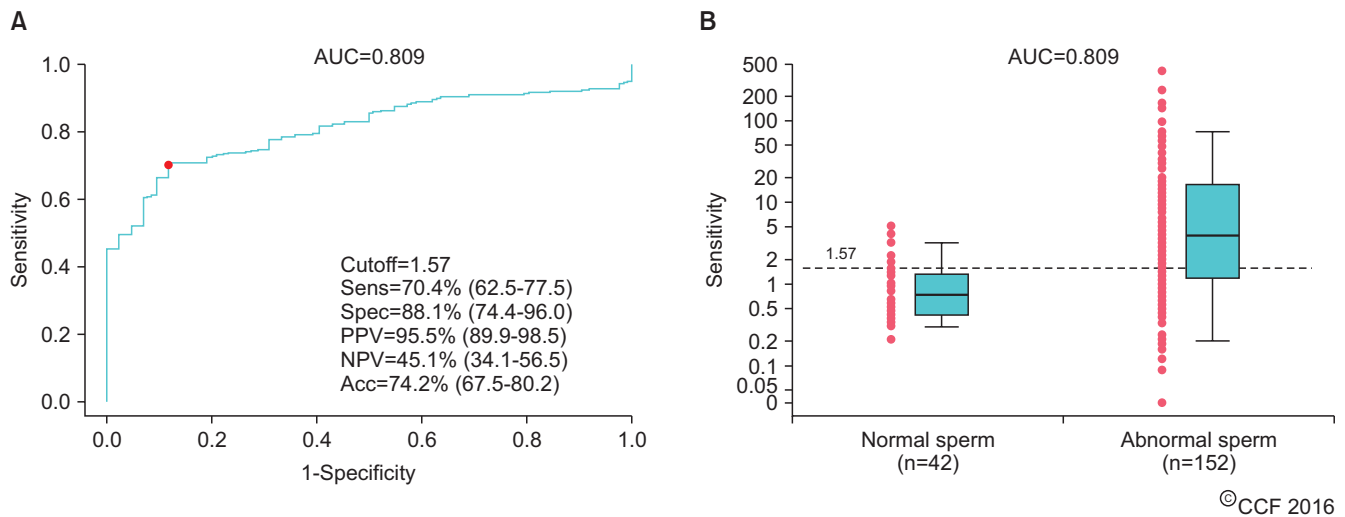
(especially concentration) is more robust in identifying OS than ORP alone.

To test the relationship between ORP and semen parameters over time, 28 infertile patients underwent repeat semen analyses and ORP measurements at baseline and after 3–5 months. At follow-up, sperm concentration, total motility, and ORP had significantly improved (Supplementary Table 2). This shows that sperm concentration and total motility increase as ORP decreases. Lastly, whether ORP can be used to monitor male infertility due to an infectious agent or inflammatory condition remains unclear. Leukocytes were present in 9 infertile semen samples as verified by the Endtz test and were treated with 200-mg doxycycline daily for 2–3 weeks. Although leukocytes were essentially eliminated, ORP did not show a statistically significant decline.

4. ORP in abnormal semen parameters regardless of fertility status

Agarwal et al. [56] then evaluated the ability of additional combinations of semen parameters and ORP to identify OS regardless of fertility status. The authors also compared ORP levels among different categories of abnormal semen parameters arranged by the 2010 WHO criteria. This study consisted of 15 healthy controls with proven fertility and 293 infertile patients. Comparing infertile patients with controls, there were significant differences in all semen parameters (p<0.05), except for ORP. However, as expected, ORP was negatively correlated with sperm concentration and motility (p<0.0001). Afterwards, healthy and infertile semen samples were grouped altogether and categorized

based on abnormal semen parameters (oligozoospermic [OZ], asthenozoospermic [AZ], teratozoospermic [TZ], and oligoasthenoteratozoospermic [OAT]). Normal semen samples were labeled normozoospermic (NZ).

ORP levels were significantly higher in the groups with abnormal semen parameters than in the NZ samples (p<0.0001). Also, ORP was highly predictive of AZ due to the high specificity (86.3%) and PPV (75.3%). This was not the case in regards to TZ. Interestingly, although ORP had a high specificity in predicting OAT, it had a low PPV. This means that an ORP value above the proposed OAT cutoff did not definitively indicate the presence of OAT due to the presence of many false positives. Similar to prior research findings, ORP was best at predicting OZ with a cutoff of 2.63 mV/10^6 sperm/mL 81.5% sensitivity, 92.7% specificity, 89.1% PPV, 87.2% NPV and AUC=0.919.

Lastly, an ORP cutoff of 2.7 mV/10^6 sperm/mL can detect at least 2 abnormal semen parameters achieving 64.6% sensitivity, 83.9% specificity, 75.7% PPV, 75.4% NPV and AUC=0.809 (p<0.0001). With this data, clinicians may use a combination of ORP and 2 other abnormal semen parameters to help identify OS rather than using ORP alone. The study results confirmed that ORP measurement is a reproducible test that is highly predictive of abnormal semen parameters regardless of fertility status. Identifying ORP provides a quick composite picture of OS status and abnormal semen parameters in just 1 patient visit. Therefore, the need for multiple follow-ups with patients are not necessary.

5. Reproducibility and reliability of ORP on a global scale

To further test ORP's consistency, a number of studies have used the MiOXSYS System in different ethnic populations at different geographical locations. Agarwal et al. [57] conducted a multicenter retrospective study that compared data from 2 andrology laboratories located in the USA and Qatar for 12 months. Cleveland Clinic, Cleveland, USA, recruited 51 healthy fertile controls and 194 infertile patients. A large tertiary hospital located in Doha, Qatar, recruited 50 fertile controls and 400 infertile patients. The sample population was analyzed as a combined dataset and as individual datasets according to institution. In both data sets and the combined dataset, sperm concentration, total motility, progressive motility, and normal morphological forms were significantly lower and ORP significantly higher in the infertile patients than in the controls (p<0.05). Between the infertile patients of the Cleveland Clinic and Doha, there were no significant differences in ORP or semen parameters except for progressive motility and morphology. Between control patients of the Cleveland Clinic and Doha, there was only a significant difference in morphology (p=0.004).

Data from the combined population and combined number of infertile patients of both institutions were divided into normal and abnormal semen groups according to the 2010 WHO semen parameters. ORP was elevated in both abnormal semen groups (p<0.001) (Tables 2, 3). When combining both study populations, ROC curve analysis generated an ORP cutoff value of 1.42 mV/10^6 sperm/mL that was able to differentiate fertile from infertile semen groups with 60.6% sensitivity, 74.3% specificity, 93.3% PPV, 24.3% NPV, and 62.6% accuracy (Supplementary Fig. 8). This ORP value persisted even with variations of baseline characteristics such as smoking status, leukocytospermia, age, body mass index, and number of abstinence days.

Arafa et al. [58] provided supporting data from Qatar through a prospective study of 365 infertile patients and 50 fertile controls. Decreased semen parameters in all categories and elevated ORP levels were found in the infertile patients (p<0.001) when compared with fertile controls. ORP also showed no significant correlation with age, BMI, or days of sexual abstinence despite differences in those baseline demographics. ROC curve analysis provided an ORP cutoff of 1.38 mV/10^6 sperm/mL to differentiate normal from abnormal semen quality with a 63.3% sensitivity, 87.8% specificity, 97.6% PPV, 23.2% NPV, and 66% accuracy. A cutoff of 1.41 mV/10^6 sperm/mL was able to distinguish fertile from infertile men with an accuracy that was better than that of other WHO semen parameters except for progressive motility—a parameter that is quite subjective due to its high intra- and interindividual variability (Table 4).

Table 2. Semen parameters and oxidation-reduction potential (ORP) in subjects (n=547) with at least one abnormal semen parameter for combined dataset of Cleveland Clinic and Doha study populations

Variable	Normal sperm (n=114)	Abnormal sperm (n=433)	p-value
Volume (mL)	3 (2–3.8)	3 (2–4)	0.91
Concentration (10^6 sperm/mL)	55.3 (40–80)	20 (5–40.8)	<0.001
Total motility (%)	64.5 (62–72)	40 (20–53)	<0.001
Progressive motility (%)	35 (32–40)	10 (0–20)	<0.001
Morphology (normal form%)	13 (9–18.5)	3 (1–6)	<0.001
ORP (mV/10^6 sperm/mL)	0.85 (0.51–1.14)	2.27 (1.09–8.77)	<0.001

Values are presented as median (interquartile range).
Adapted from Agarwal et al. Andrology 2017;5:939-45 [57], permission of John Wiley and Sons.

Table 3. Semen parameters and oxidation-reduction potential (ORP) in infertile patients (n=497) with at least one abnormal semen parameter for combined dataset of Cleveland Clinic and Doha study populations

Variable	Normal sperm (n=90)	Abnormal sperm (n=407)	p-value
Volume (mL)	3 (2–3.75)	3 (2–4)	0.21
Concentration (10^6 sperm/mL)	54.8 (40–80)	19 (4.96–39)	<0.001
Total motility (%)	65 (62–72)	40 (20–52)	<0.001
Progressive motility (%)	35 (32–43)	9 (0–20)	<0.001
Morphology (normal form%)	15 (9–20)	3 (1–6)	<0.001
ORP (mV/10^6 sperm/mL)	0.83 (0.51–1.11)	2.49 (1.15–10.16)	<0.001

Values are presented as median (interquartile range).
Adapted from Agarwal et al. Andrology 2017;5:939-45 [57], permission of John Wiley and Sons.

Table 4. Predicting semen samples from infertile patients and from fertile donors

Variable	sORP (Youden's Index)	Total number (WHO cutoff)	Total motility (WHO cutoff)	Progressive motility (WHO cutoff)	Normal morphology (WHO cutoff)
Cutoff for infertility	>1.41 mV/10^6/mL	39×10^6	40%	32%	<4%
Sensitivity	57.3% (52.0–62.4)	28.2% (23.7–33.1)	40.8% (35.7–46.1)	88.0% (84.2–91.1)	50.4% (45.2–55.7)
Specificity	78.0% (64.0–88.5)	92.0% (80.8–97.8)	94.0% (83.5–98.7)	28.0% (16.2–42.5)	84.0% (70.9–92.8)
PPV	95.0% (91.2–97.5)	96.3% (90.7–99.0)	98.0% (94.3–99.6)	89.9% (86.3–92.8)	95.8% (92.0–98.2)
NPV	20.0% (14.6–26.3)	14.9% (11.1–19.4)	17.9% (13.4–23.0)	24.1% (13.9–37.2)	18.8% (13.9–24.6)
Accuracy	60%	35.9%	47.2%	80.7%	54.5%

Values are presented with 95% confidence interval.
sORP, static oxidation-reduction potential; WHO, World Health Organization; PPV, positive predictive value; NPV, negative predictive value.
Adapted from Arafa et al. Andrologia 2017 Aug 3 [58], permission of John Wiley and Sons.

By enrolling a large number of patients, these studies were instrumental in providing reliable ORP cutoffs that can identify abnormal semen samples and infertile men. Since these values are similar to the ones proposed in previous studies, measuring ORP is a reliable and reproducible technique when analyzing semen samples in diverse populations.

FUTURE DIRECTION OF OXIDATION-REDUCTION POTENTIAL CLINICAL USE

ORP's role as a surrogate marker to aid in the diagnosis of male infertility is under investigation by numerous groups. The following topics were discussed primarily through some recent abstracts presented at national and international medical conferences within the past 2 years. Many of the studies attempted to improve upon previously published papers by recruiting larger sample sizes treating leukocytospermia, monitoring ORP response to treatment, classifying patients according to etiologies of male infertility, and evaluating female factor infertility in the patients' female partners.

1. Relationship to semen parameters

There is convincing evidence that further supports the relationship between ORP and semen parameters. According to Roychoudhury et al. [65], an ORP cutoff of 1.23 mV/10^6 sperm/mL could identify over 90% of healthy semen samples which was defined as sperm that meets all of the 2010 WHO normal semen parameter criteria. In fact, Elbardisi et al. [66] found that semen with 1 or more poor parameters had higher ORP values than semen that met all of WHO criteria (p<0.05). ORP increased with abnormal semen parameters. Lastly, an ORP cutoff of 1.635 mV/10^6 sperm/mL had a 98.6% chance of predicting semen with at least 1 or more abnormal semen parameters. Toor et al. [67] observed that ORP was elevated in semen that had a low

sperm concentration, low total sperm count, and low sperm motility (p<0.05). There was also a negative correlation with ORP and these parameters (p<0.05). Lastly, Agarwal et al. [68] conducted a multicenter study at 9 institutions around the world with 2010 recruited participants. An ORP cutoff value of 1.34 mV/10^6 sperm/mL was able to identify samples with abnormal semen parameters with 58% sensitivity, 85% specificity, 96% PPV, 42% NPV, and AUC=0.757. The cutoffs and relationships observed in these studies were similar to the ones established by Cleveland Clinic researchers.

ORP's relationship to sperm morphology is inconsistent. This adds to the controversy of whether to use sperm morphology as an indicator of sperm function [69-71]. The aforementioned clinical studies found weak evidence in supporting ORP to predict teratozoospermia [55,56]. However, Arafa et al. [72] suggested that a cutoff of 3.29 mV/10^6 sperm/mL was able to reliably predict abnormal morphology with a 55.6% sensitivity, 89.1% specificity, 85.7.1% PPV, 63.1% NPV and 71% accuracy (AUC=0.90). Additionally, Ayaz et al. [73] found that although sperm morphological abnormalities were present in both fertile and infertile men, they were often found in combination with other semen parameter abnormalities in infertile semen samples. ORP also increased in infertile semen samples as the percentage of sperm neck abnormalities increased.

Total motile sperm count (TMSC) is an excellent semen parameter in predicting severity of male infertility [74,75]. Al Said et al. [76] sought to determine a correlation between ORP and TMSC, in hopes that these 2 parameters could provide additional information in the evaluation of male infertility. A significant negative correlation was found (p<0.001). Also, ROC curve analysis showed that an abnormal TMSC (>20 million) can be best predicted with an ORP cutoff of 2.34 mV/10^6 sperm/mL with a 83.5% sensitivity, 82.5% specificity, 82.9% PPV, 81.4% NPV, and 79.9% accuracy (AUC=0.9). Because of the promising data, this study advocates for concurrent measurement of TMSC and ORP

to evaluate male infertility.

2. DNA fragmentation, reproductive outcomes, and ART

Sperm DNA fragmentation (SDF) is most commonly seen in infertile men, which if not detected early, can impair fertilization, embryo development, and successful clinical pregnancy [77-80]. Arafa et al. [81] conducted a prospective study of 312 patients and reported that DNA fragmentation was negatively correlated with total/progressive motility (p<0.001) and positively correlated with abnormal morphology, ORP, and age (p<0.001). Elevated ORP levels were also seen in the semen of the high SDF group compared to that of the normal SDF group (4.03±0.61 mV/10^6 sperm/mL and 2.14±0.14 mV/10^6 sperm/mL, respectively, p<0.001).

In a cross sectional study of 1,162 patients, Majzoub et al. [82] observed a positive correlation between the percentage of abnormal sperm heads and levels of ORP (p<0.001) and SDF (p<0.001). This confirms that OS is highly implicated in sperm DNA damage, especially SDF [83-85]. In regards to reproductive outcomes, Ayaz et al. [86] observed that the clinical pregnancy rate was higher in patients in a low ORP group (<1.36 mV/10^6 sperm/mL) than in those in a high ORP group (>1.36 mV/10^6 sperm/mL) (p=0.006). For ART purposes, monitoring ORP allows for better sperm selection and preparation [87]. Overall, ORP not only detects OS-associated damage, but also provides clues on prognosis which fully informs the clinician on an individual's clinical status.

3. Varicocele

Varicocele is the most common correctable cause of male infertility. Although there are many possible mechanisms of varicocele-induced injury, OS appears to be the main culprit [88]. Agarwal and colleagues observed that ORP levels were higher and semen parameters (concentration, total motility, and morphology) were lower in varicocele patients than in healthy controls (p=0.001) [89,90]. ORP was also negatively correlated with concentration, total motility and morphology (p=0.001) [90]. Currently, there is no consensus on the differences of ORP among grades of varicocele—one study found no difference whereas another study stated that patients with a grade 3 varicocele had the highest levels [90,91]. Lastly, when compared to patients of idiopathic infertility, varicocele patients had significantly lower sperm concentration, fewer normal morphologic forms, and higher seminal ORP (p<0.05) [92].

4. Leukocytospermia

Genitourinary infections are a major contributor to OS-induced male infertility. Accumulated leukocytes in the seminal plasma (leukocytospermia) increase ROS production and lead to abnormal semen parameters [93,94]. In a preliminary study, Sikka and colleagues [95,96] proposed monitoring ORP as an indicator of OS in leukocytospermia because ORP values paralleled the levels of certain biomarkers of active inflammation such as the Toll-like receptor 4 and cyclooxygenase 2.

MANAGEMENT OF ELEVATED OXIDATION-REDUCTION POTENTIAL

A clinician should measure ORP in all patients who present to the infertility clinic as an adjunct to the basic semen analysis. As demonstrated in clinical studies, the excellent results show that ORP can provide valuable information about sperm function and a man's fertilization capabilities. This highlights ORP measurement as an important ancillary tool for male infertility evaluation. ORP can be measured to assess patients' initial OS status and to help guide therapeutic interventions. Theoretically, ORP should decrease when the underlying cause of OS is eradicated. Varicocelectomy, antioxidant supplementation, antibiotic treatment, and lifestyle modifications are examples of treatments that may be beneficial in alleviating OS and improving reproductive outcomes [97-100]. This suggests that ORP measurement is perhaps most beneficial for patients with varicocele, idiopathic infertility, and OS-inducing lifestyle habits. Nonetheless, further studies are needed to determine if ORP can be used to longitudinally monitor treatment progress in patients with specific clinical conditions.

CONCLUSIONS

Male infertility is a multifactorial condition in which OS plays a central role. Newer measures such as ORP represent an objective and accurate method that can reliably identify abnormal semen quality and differentiate fertile from infertile men. The ORP test is also a cost-effective and convenient option for patients undergoing evaluation for male factor infertility. ORP measurement has not yet been incorporated into standard clinical practice due to an incomplete understanding of the clinical indications. Its ability to discriminate across different clinical conditions and to monitor therapeutic effectiveness remains unclear. Also, the current evidence is not yet strong enough to

advocate for widespread use of ORP as a stand-alone test. Thus, ORP measurement by the MiOXSYS System provides a novel diagnostic method that may be used in conjunction to routine semen analysis with hopes of positively affecting the lives of men burdened by infertility.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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SUPPLEMENTARY MATERIALS

Scan this QR code to see the supplementary materials, or visit http://www.icurology.org/src/sm/icurology-58-385-s001.pdf.

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Supplementary Table 1. Summary of the commonly used techniques to evaluate seminal oxidative stress

Technique	Instrument	Advantages	Disadvantages
ORP	MiOXSYS	Provides a snapshot of the redox balance in real time Levels of all oxidants and reductants are measured Less time consuming Inexpensive materials Simple methodology Both fresh and frozen semen and seminal plasma can be measured	Semen age, high viscosity, and repeated centrifugation may alter results of measurements
ROS by chemiluminescence	Luminometer	Robust chemiluminescence High sensitivity and specificity Intracellular and extracellular ROS are detected	15–30 minutes to yield test results Cost and size of equipment Semen age, volume, repeated centrifugation, temperature and background luminescence may alter results of measurements
TAC	Colorimeter Luminometer	Reliable and predictive of antioxidant capacity Total antioxidants in seminal plasma measured	Cannot differentiate the amounts of enzymatic and nonenzymatic antioxidants independently Long duration of inhibition time Cost of microplate readers
ROS-TAC	Statistical analyses	Superior to ROS or TAC alone	Calculated through statistical analyses Does not directly measure ROS or TAC
MDA	Colorimeter and fluorometer for MDA-TBA adduct HPLC	Assesses lipid peroxidation	Rigorous controls required Nonspecific test Only detects *post hoc* damage

ORP, oxidation-reduction potential; ROS, reactive oxygen species; TAC, total antioxidant capacity; MDA, malondialdehyde; TBA, thiobarbituric acid; HPLC, high performance liquid chromatography.
Adapted from Agarwal et al. Ther Adv Urol 2016;8:302-318 [22].

Supplementary Table 2. Semen parameters and oxidation-reduction potential (ORP) changes in all patients (n=28) who had repeated tests done over a period of 16.8±7.7 weeks

Variable	1st sample	2nd sample	p-value
Concentration (10^6 sperm /mL)	7.2 (2.93–20.73)	10.55 (5.22–33.3)	0.019
Total motility (%)	32 (17.5–47.5)	42.5 (29.25–53.75)	0.008
Morphology (normal form %)	2 (1–5.5)	2 (1.25–4.75)	0.57
ORP (mV/10^6 sperm/mL)	6.08 (2.63–15.92)	2.06 (0.7–9.16)	0.007
Patients treated with doxycycline (n=9)			
Endtz (M/mL)	0.8 (0.4–2.3)	0 (0–0.4)	0.024
ORP (mV/10^6 sperm/mL)	0.77 (0.49–1.05)	0.62 (0.49–0.74)	0.086

Values are presented as median (interquartile range).
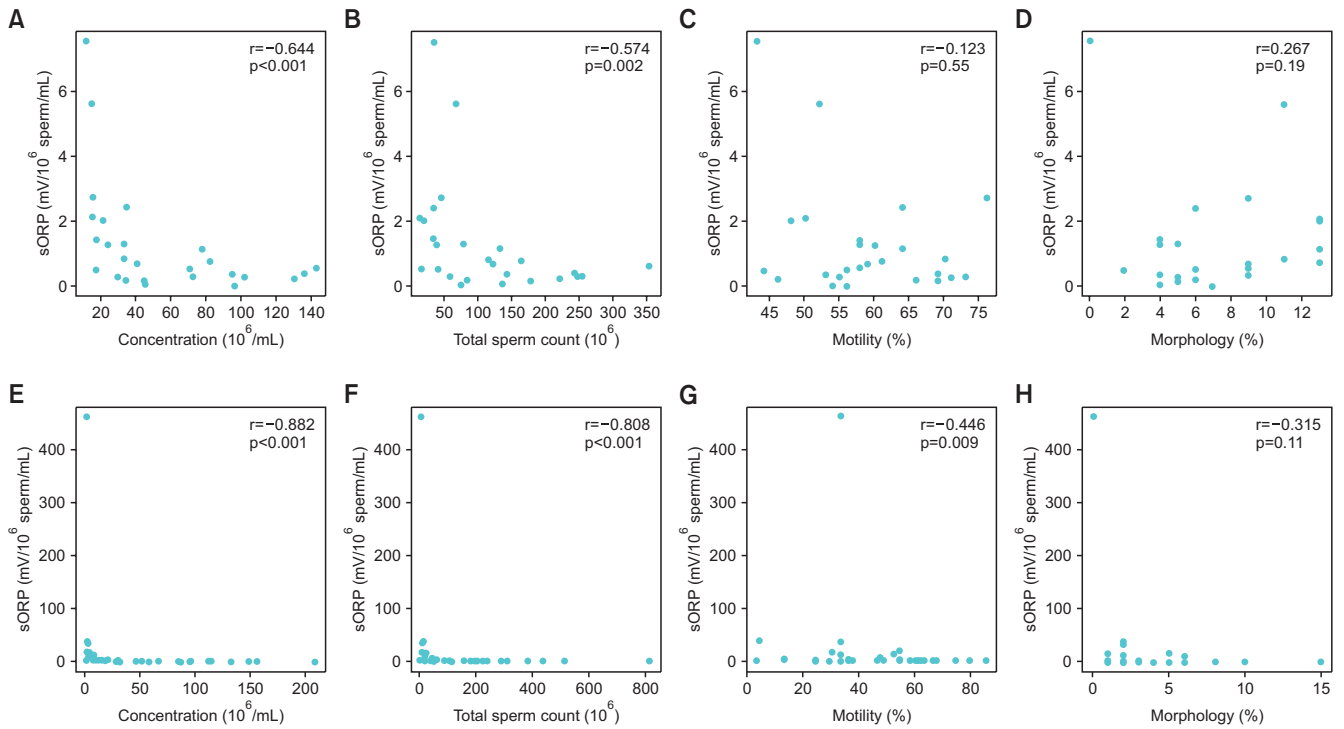Adapted from Agarwal et al. Urology 2017;104:84-9 [55], permission of Elsevier.
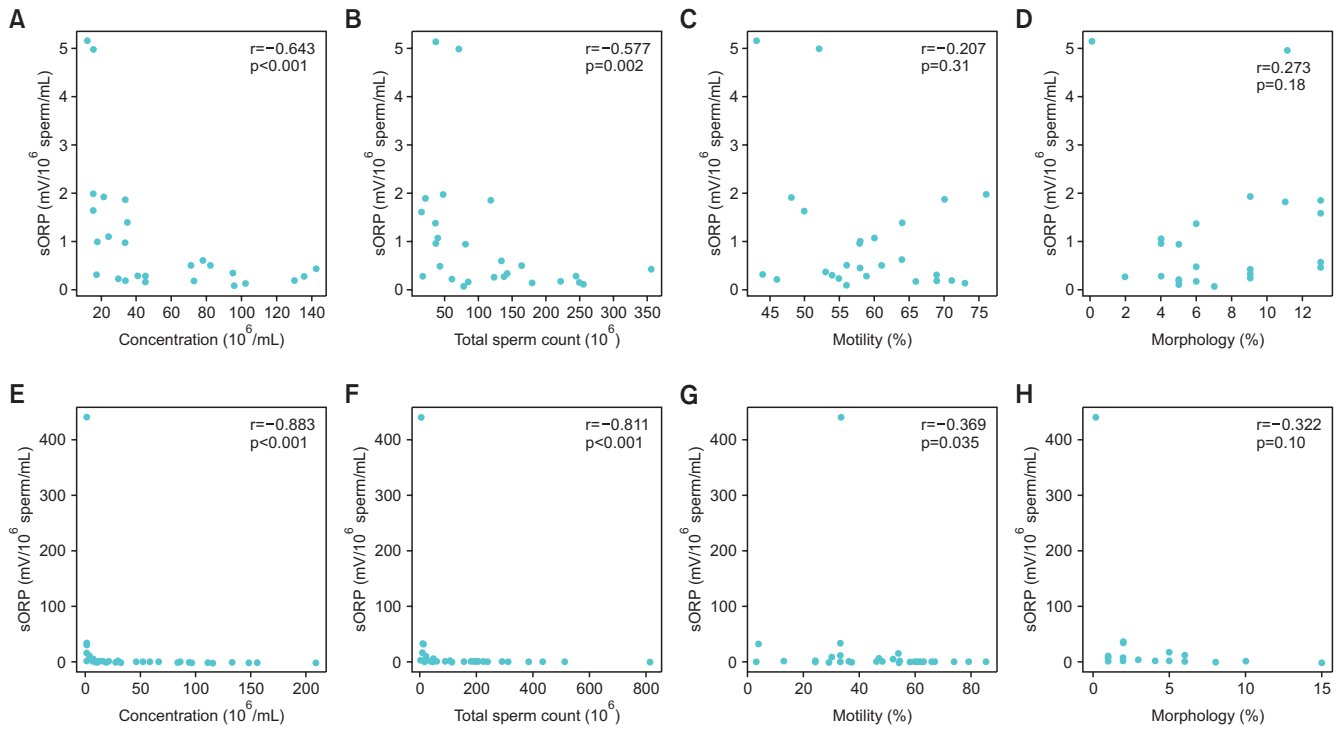
Supplementary Fig. 1. Correlation of semen static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) with semen parameters at 0 minutes in healthy controls (A, concentration; B, total sperm count; C, motility; D, morphology) and infertile patients (E, concentration; F, total sperm count; G, motility; H, morphology). Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
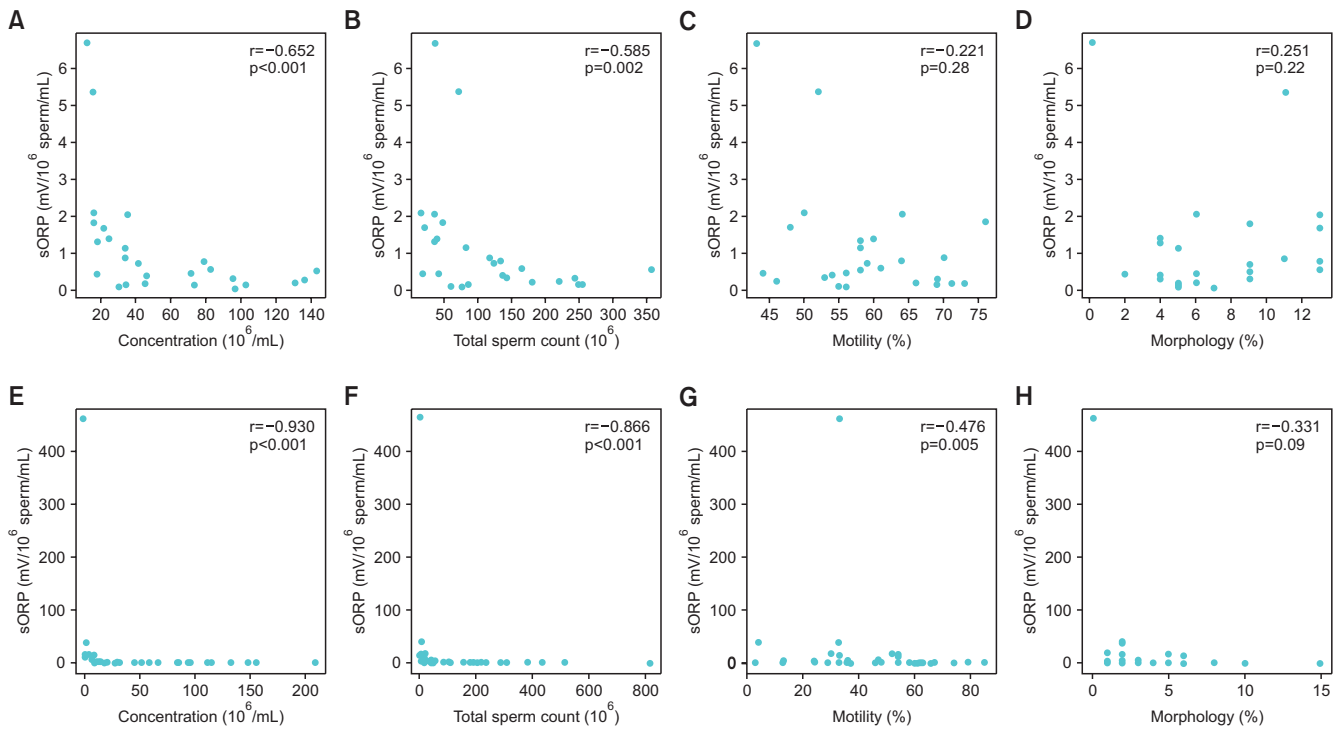
Supplementary Fig. 2. Correlation of seminal plasma static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) with semen parameters at 0 minutes in healthy controls (A, concentration; B, total sperm count; C, motility; D, morphology) and infertile patients (E, concentration; F, total sperm count; G, motility; H, morphology). Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
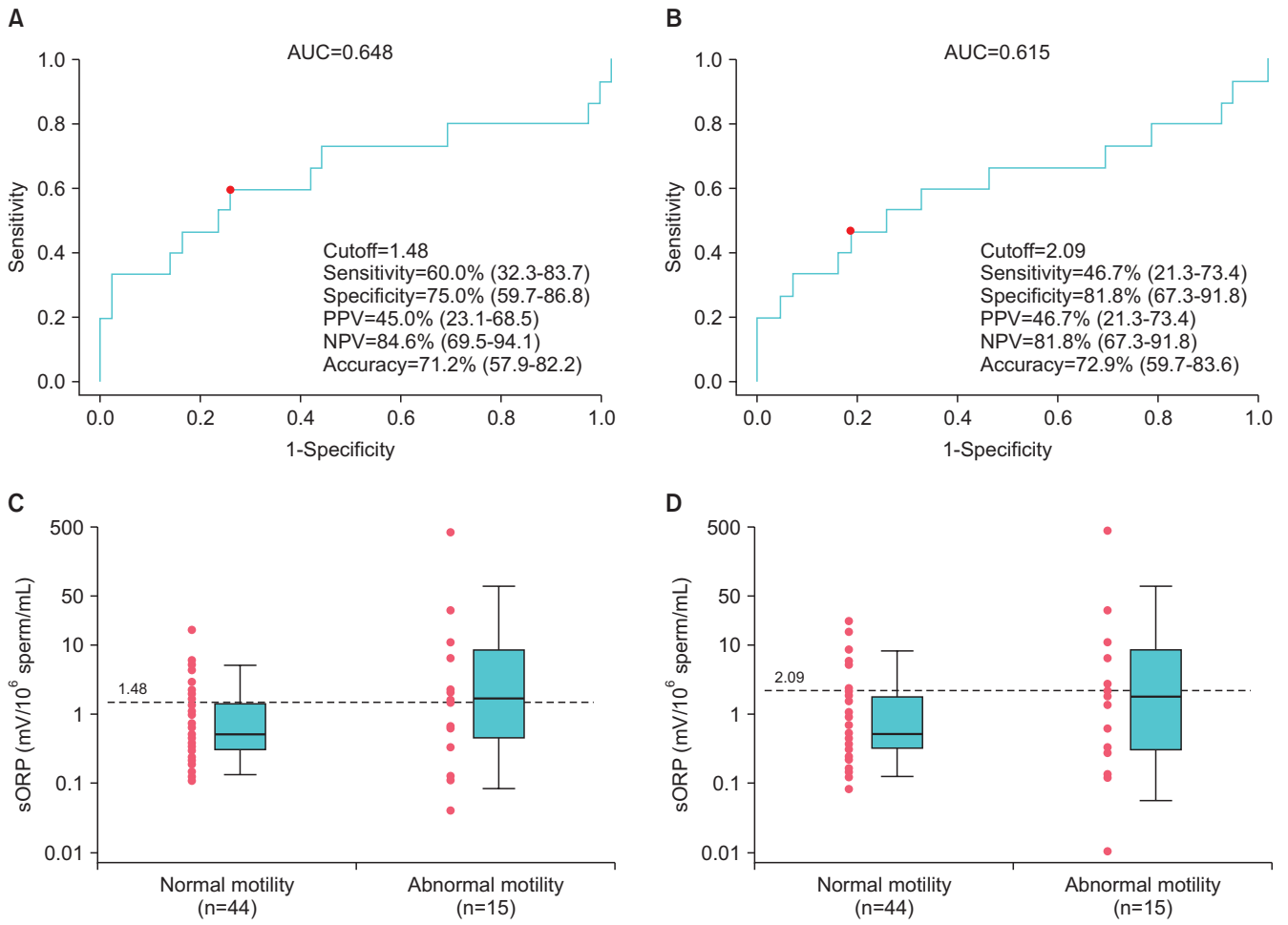
Supplementary Fig. 3. Correlation of semen static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) with semen parameters at 120 minutes in healthy controls (A, concentration; B, total sperm count; C, motility; D, morphology) and infertile patients (E, concentration; F, total sperm count; G, motility; H, morphology). Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
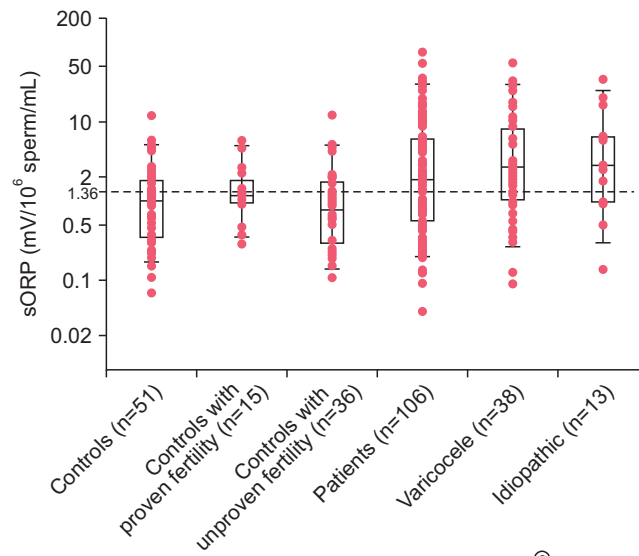
Supplementary Fig. 4. Correlation of seminal plasma static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) with semen parameters at 120 minutes in healthy controls (A, concentration; B, total sperm count; C, motility; D, morphology) and infertile patients (E, concentration; F, total sperm count; G, motility; H, morphology). Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
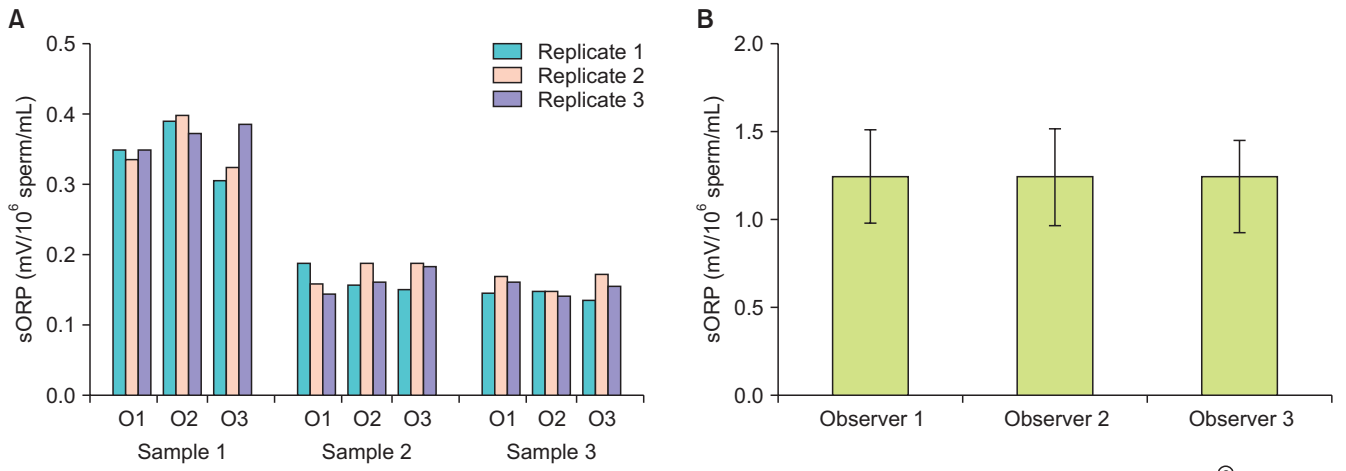
A

AUC=0.648

Cutoff=1.48
Sensitivity=60.0% (32.3-83.7)
Specificity=75.0% (59.7-86.8)
PPV=45.0% (23.1-68.5)
NPV=84.6% (69.5-94.1)
Accuracy=71.2% (57.9-82.2)

B

AUC=0.615

Cutoff=2.09
Sensitivity=46.7% (21.3-73.4)
Specificity=81.8% (67.3-91.8)
PPV=46.7% (21.3-73.4)
NPV=81.8% (67.3-91.8)
Accuracy=72.9% (59.7-83.6)

C

D

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Supplementary Fig. 5. Receiver operating characteristic curve establishing the cutoff in semen (A) and seminal plasma (B). Distribution of static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) in subjects with normal and abnormal motility in semen (C) and seminal plasma (D), suggesting a criterion for sORP in distinguishing semen quality based on good (≥40%) and poor (≤40%) motility. AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

Supplementary Fig. 6. Distribution of static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) in (1) normal healthy controls; (2) controls with proven fertility; (3) controls with unproven fertility; (4) infertile patients; (5) infertile patients presenting with a clinical varicocele; and (6) those with idiopathic infertility. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.

Supplementary Fig. 7. Static oxidation-reduction potential (sORP) (mV/10^6 sperm/mL) across samples and observers showing (A) Intraobserver reliability by observing the replicate sORP measures for each of the three samples. Most replicates were similar to each other and across the 3 observers (01–03). (B) Interobserver reliability by comparing sORP-values across observers. The mean sORP for each observer was equivalent with similar standard error of the mean, suggesting that all observers obtained similar sORP-values for each of the 10 samples tested, which were measured in 4 replicates. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.
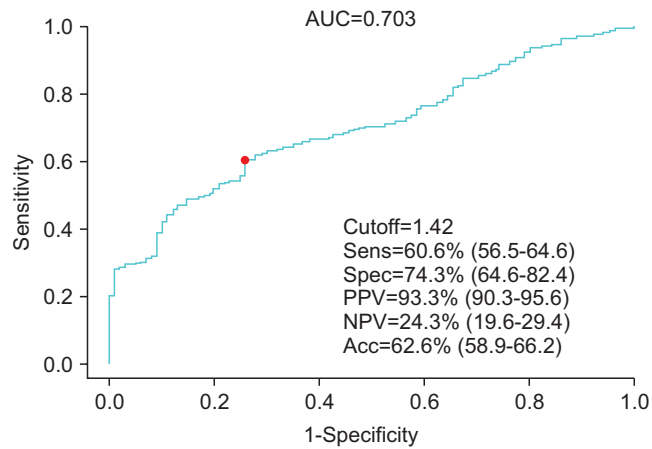
Supplementary Fig. 8. Receiver operating characteristic curve of oxidation-reduction potential (ORP) (mv/10^6 sperm/mL) in distinguishing infertile patients from healthy controls in the combined dataset (Cleveland Clinic and Doha, Qatar). Acc, accuracy; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2013-2017.