Infertility

Clinical Relevance of Oxidation-Reduction Potential in the Evaluation of Male Infertility

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OBJECTIVE
To evaluate (1) the relationship between oxidation-reduction potential (ORP) and abnormal sperm quality, and (2) the changes in ORP and sperm parameters over time, in search of a potential surrogate marker of poor sperm quality that may assist in the diagnosis of oxidative stress-related male infertility.

MATERIALS AND METHODS
A total of 194 infertile men were included and 28 patients were identified to have repeated semen analyses and ORP measurements. The semen samples obtained were categorized into normal and abnormal sperm parameters based on the World Health Organization’s fifth edition guidelines. Wilcoxon tests were used to compare the results of different groups. Correlations were analyzed by the Spearman rank-order correlation and receiver operating characteristic analysis was used to estimate optimal ORP cutoffs for identifying abnormalities.

RESULTS
ORP levels were significantly elevated in semen samples with abnormal sperm parameters. ORP at a cutoff of 1.57 (mV/10⁶ sperm) was able to detect at least 1 abnormal sperm parameter with a sensitivity of 70.4% and a specificity of 88.1%. ORP at a cutoff of 2.59 (mV/10⁶ sperm) had the highest predictive value in detecting oligozoospermia with 88% sensitivity and 91.2% specificity. The increases in sperm concentration and motility in patients tested for semen analysis at 2 consecutive time intervals were related to a decline in ORP levels.

CONCLUSION
ORP is a reliable method in predicting poor sperm quality. The introduction of ORP in male infertility evaluation may help overcome the high technical variability of semen analysis and assist in the diagnosis of oxidative stress-related infertility. UROLOGY 104: 84–89, 2017. © 2017 Elsevier Inc.

Infertility is a global public health issue, affecting approximately 48.5 million couples worldwide.1,2 Of note, the male factor attributes to 20%-70% of these cases.2 As part of the male infertility evaluation, most clinicians rely on the “gold standard” test, conventional semen analysis, as a surrogate measure of a man’s fertilizing ability.3 However, semen analysis has a poor predictive ability due to its high technical (intra- and interassay) variability and the lack of stability of individual sperm parameters.4 Furthermore, semen analysis provides no information about the sperm function.3 Hence, new markers have been developed to assess the sperm quality and to detect possible etiology of male infertility. Among these markers, oxidative stress (OS) markers are gaining popularity as OS is a well-recognized key mediator in the pathogenesis of male infertility.5

OS occurs when there is an excessive production of reactive oxygen species (ROS) or a substantial decrease in antioxidants. ROS are radical or nonradical oxygen metabolites that are highly reactive, whereas antioxidants are enzymatic or nonenzymatic substances that counteract ROS. Low physiological amounts of ROS are required to trigger essential sperm functions such as capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion.6 However, when OS occurs, it leads to lipid peroxidation of sperm membranes and intracellular lipids and proteins, aggravates apoptosis, and results in DNA damage.7 Such effects are harmful to sperm cells, resulting in poor sperm quality and consequently reduced fertility.8

Conventional methods of OS measurements include chemiluminescent ROS assay, total antioxidant capacity (TAC) test, ROS-TAC score, and malondialdehyde measurement. Although these methods provide useful information, these tests are mostly time-consuming and require large and expensive equipment as well as statistical modeling.9 Recently, a novel technology measuring oxidation-reduction potential (ORP), the MiOXSYS analyzer, has...
been developed. The MiOXSYS system is fast and simple to use. The ORP measured, or known as the redox potential, is a measure of transfer of electrons. Instead of measuring ROS or antioxidant separately, ORP provides a comprehensive measure of both oxidants and antioxidants concurrently, representing the oxidative state in real time. ORP levels have been shown to correlate negatively with sperm parameters, distinguishing infertile men from normal controls.10

The objectives of our study were to evaluate (1) the relationship between ORP and abnormal sperm parameters in infertile men and (2) the changes in ORP levels over time to determine whether ORP can be a potential surrogate marker of poor sperm quality, and if so, (3) to establish a cutoff value of ORP to distinguish abnormal from normal sperm parameters.

MATERIALS AND METHODS

Subject Selection

Semen samples were obtained from 49 normal healthy controls and 194 infertile patients who attended the male infertility unit at Cleveland Clinic, Cleveland, Ohio, from August 2015 to August 2016; and a total of 28 patients were identified to have repeated semen analyses and ORP measurements. Ethical clearance from the local institutional review board and informed consent from the patients were obtained before analyzing the samples.

The control group (N = 49) was composed of healthy men with proven (n = 15) and unproven (n = 34) fertility who had normal semen parameters based on the World Health Organization (WHO) fifth edition guidelines.11

Semen samples obtained from the infertile patients (N = 194) were categorized into normal and abnormal sperm parameter groups using the WHO fifth edition guidelines.11 The abnormal sperm parameter groups had at least one of the following abnormal sperm parameters: a sperm concentration of <15 x 10⁶ sperm/mL (oligozoospermia), a total motility of <40% (asthenozoospermia), or a normal morphology of <4% (teratozoospermia). Additionally, the presence of varicoceles in the infertile patients was documented by a trained male infertility specialist or urologist.

In all subjects who came for a second semen analysis and ORP test (N = 28), a total of 2 semen samples were collected, at baseline (first sample) and after 3-5 months (second sample). Nine of these patients were Endtz positive and were treated with doxycycline for 2-3 weeks at a dosage of 200 mg/day. The majority of the remaining patients (n = 19) were prescribed with either clomiphene citrate (n = 4), antioxidants (n = 5), or a combination of both (n = 5).

Semen Analysis

Semen specimens were collected in sterile containers after a period of 48-72 hours of sexual abstinence. After liquefaction at 37°C for 20 minutes, manual semen analysis was performed to determine the sperm parameters according to WHO criteria.11 In addition, an Endtz test to identify peroxidase-positive cells was performed. An aliquot of 5 μL of the sample was loaded on a MicroCell counting chamber (Vitrolife, San Diego, CA) and observed under a phase-contrast microscope set at 20x magnification for sperm concentration and motility. For sperm morphology, Diff-Quik Staining Method (Baxter Healthcare Corporation, Inc., McGaw Park, IL) was employed. The air-dried smears were fixed and stained and a total of 200 sperm were scored. A percentage of normal forms of ≥4% were used as a normal cutoff.

Measurement of ORP

ORP was measured in millivolt using the galvanostat-based technology, the MiOXSYS system (Aytu BioScience, Englewood, CO). A 30 μL aliquot of liquefied semen was added to the preinserted sensor, and the static ORP was measured by applying a low-voltage current and was eventually normalized to the seminal sperm concentration. The whole process took less than 4 minutes. The ORP measured reflects a “snapshot” of the current balance of the redox system with a higher ORP level suggesting a higher state of OS.

Statistical Analysis

Data are presented as median (25th and 75th percentiles). A P value of <.05 was considered as statistically significant for pairwise group comparison using Wilcoxon signed-rank test and for unpaired group comparison using Wilcoxon rank-sum test. The correlations between the ORP and sperm parameters were analyzed by the Spearman rank-order correlation. The best cutoff value, sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve were calculated by receiver operating characteristic analysis. Box-and-whisker plots were used to demonstrate the distributions of the ORP (mV/10⁶ sperm) cutoff between normal and abnormal sperm parameters.

RESULTS

The comparison of sperm parameters and ORP levels between the controls (N = 49) and the infertile men (N = 194) are summarized in Supplementary Table S1. The sperm concentration, total motility, and normal morphology forms were significantly lower in infertile men than in the controls (P < .001). On the contrary, the ORP levels were significantly elevated in infertile men (P < .001).

The correlations of ORP and sperm parameters of all infertile men (N = 194) are shown in Supplementary Figure S1A-C. The results of ORP levels in abnormal sperm parameter groups are summarized in Table 1. ORP levels were found to be negatively correlated with sperm parameters (concentration, motility, and morphology) and were significantly elevated in all abnormal sperm parameters groups (oligozoospermia, asthenozoospermia, and teratozoospermia).

Patients in the oligozoospermia group appeared to have the highest ORP levels compared to those with asthenozoospermia or teratozoospermia. Of note, sperm motility in 1 sample and the morphology of 20 samples were not calculated as the sperm counts were too low for accurate counting of motility or morphology. At a cutoff value of 1.57 (mV/10⁶ sperm), ORP was able to detect at least 1 abnormal sperm parameter with 70.4% sensitivity, 88.1% specificity, and 95.5% positive predictive value (P < .001) (Fig. 1A). The distributions of ORP in normal and abnormal sperm parameters at this cutoff value are shown in Figure 1B. Comparison of sensitivities, specificities, and predictive values of different ORP cutoff values in different sperm parameters indicated that these values were highest.
in the oligozoospermia group (P < .001) (Fig. 2, Supplementary Table S2).

Out of the 194 infertile men, 44 (22.7%) had unilateral and 29 (14.9%) had bilateral varicoceles of variable clinical grades. There were no significant differences in ORP levels between patients with and without varicoceles (P = .72, Supplementary Table S3).

In patients with repeated semen analyses and ORP measurements (n = 28), the mean ± standard deviation of the interval between the first and second semen samples was 16.8 ± 7.7 weeks. There were 20 oligozoospermic, 18 asthenozoospermic, and 18 teratozoospermic specimens identified on the first semen analysis, whereas 17 oligozoospermic, 13 asthenozoospermic, and 17 teratozoospermic samples were reported on the repeat semen analysis (second sample). Changes in sperm parameters and ORP are presented in Table 2. Sperm concentration and motility significantly increased over this period of time, whereas there was no significant difference for morphology. ORP levels, on the other hand, decreased over the same intervals. Nine out of 28 patients (32%) had a positive Endtz test result (≥0.2 M/mL) and were treated with an antibiotic (doxycycline 200 mg/day) for 2-3 weeks as an empirical treatment. The white blood cell (WBC) concentrations decreased significantly (63%, P = .024) following the antibiotic treatment, with a concomitant decrease (56%) in ORP levels although statistically not significant (P = .086).

**DISCUSSION**

Besides detailed history and physical examination, routine semen analysis is still the cornerstone of male infertility evaluation. However, previous studies have shown an overlap in semen parameter values between fertile and infertile men based on the WHO fourth edition semen analysis guideline. Furthermore, with the change in the WHO guideline in 2010, which incorporates lower reference values than earlier guidelines, there have been concerns that the number of infertile men will be under-represented. Although being a cost-effective and noninvasive test, semen analysis has a high technical and marked biological variability in semen sample characteristics between regions and individuals, and even between consecutive samples from the same individuals. In view of these limitations of semen analysis and its inability to provide information regarding sperm function, it is imperative to develop further complementary tests to provide a more comprehensive assessment of male infertility.

**Table 1.** ORP values (mV/10⁶ sperm) in groups of patients with abnormal sperm parameters

<table>
<thead>
<tr>
<th>ORP Value in Abnormal Semen Parameter</th>
<th>ORP Value in Normal Semen Parameter</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>11.55 (4.8, 31.34) (n = 92, oligozoospermia*)</td>
<td>0.94 (0.43, 1.56) (n = 102, normal concentration)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>6.91 (0.44, 2.76) (n = 102, asthenozoospermia†)</td>
<td>1.27 (0.44, 2.76) (n = 9, normal motility)</td>
<td>.001</td>
</tr>
<tr>
<td>2.42 (1.06, 10.28) (n = 95, teratozoospermia‡)</td>
<td>1.2 (0.5, 3.81) (n = 79, normal morphology)</td>
<td>.003</td>
</tr>
<tr>
<td>3.76 (1.19, 16.4) (n = 152, at least 1 abnormal semen parameter)</td>
<td>0.74 (0.41, 1.3) (n = 42, normal semen parameter)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

ORP, oxidation-reduction potential. Values are presented as median (25th and 75th percentiles). * Concentration <15 × 10⁶/mL. † Total motility <40%. ‡ Morphology, normal form <4%.
ORP cutoff of 2.59 (mV/10^6 sperm). This finding suggests that OS is likely a key mechanism of reduced sperm count in patients with oligozoospermia, thus confirming earlier studies using ROS measurements as parameter suggesting such relationship.\textsuperscript{21,22}

Oligozoospermia is one of the most common abnormal semen conditions in infertile men and is often a marker of general medical and future health problems in men.\textsuperscript{23,24} Keeping all these in mind, it is important to accurately diagnose oligozoospermia early to plan for further investigation and treatment. The high sensitivity and specificity of ORP found in our study therefore may potentially be able to help in screening and even diagnosing patients with oligozoospermia. Hence, the possible role of ORP in monitoring the progression of these conditions following medical or surgical treatment should be further explored. If ORP is eventually established as a means of monitoring sperm count and therapeutic response, the potential therapeutic benefits and roles of antioxidants could be clarified.

Lastly, our study also showed that the ORP levels declined with increases in sperm concentration and motility in patients who had repeated semen analyses and ORP levels performed, demonstrating that ORP can serve as an indicator of sperm quality over time. The lack of significant improvement in sperm morphology was possibly related to the high inter- and intraobserver variabilities resulting from the technical difficulty of sperm morphology examination.\textsuperscript{19,29} The other possible explanation is that, as the interval between the two semen sample measurements was only 16.8 weeks, the interval between collection periods may have been too short for a significant change in morphology to be observed. Additionally, as sperm morphology is a relatively stable parameter, it poses a question as to whether damage to the morphology is reversible.

We were intrigued to find out the reason behind the improvements in the sperm concentration and motility. What we found was that most of these patients (9 out of 28, 32%) had a positive Endtz test result and were empirically treated with doxycycline. Such treatment resulted in both decrease in seminal WBC concentrations and ORP levels, although statistically insignificant, which is perhaps due to the small sample size. The concomitant reduced ORP along with WBC concentrations would further support the finding that leukocytospermia is a major source of ROS as was demonstrated using ROS measurements.\textsuperscript{30} Due to the small sample size of the present study, we are unable to draw any conclusion whether ORP can potentially be used as a test to monitor male infertility related to inflammatory or infectious disease in the genitourinary tract.

Our study has some limitations: (1) study groups were heterogeneous as we did not classify the infertile patients based on their clinical diagnoses or by the number of abnormal sperm parameters; (2) the changes in ORP studied over time included a smaller number of patients; (3) we did not eliminate semen samples that were positive for leukocytospermia; and (4) female factors were not included as the time frame of the study was relatively short (1 year), and not all patients’ female partners had finished undergoing infertility evaluations.

In conclusion, we advocate the use of ORP as a novel method in male infertility evaluation as it is able to identify the major etiological factor, OS, of male infertility along with predicting abnormal sperm quality. It is a much more
reproducible method for measuring OS and has many advantages over other OS measurements. Our study has shown that ORP levels were able to predict poor sperm quality at a time and over time. A cutoff value of 1.57 (mV/10⁶ sperm) was able to detect at least 1 abnormal sperm parameter with 70.4% sensitivity and 88.1% specificity.

Furthermore, ORP is best in predicting oligozoospermia, which can potentially be used in different clinical settings as a screening or confirmatory test. The use of ORP as a standardized OS measurement in future research may help to make comparison of different studies easier, which can facilitate the development of effective intervention for

![Figure 2](image)

**Figure 2.** AUC-receiver operating characteristic of the oxidation-reduction potential in different groups of abnormal sperm parameters: (A) oligozoospermic group, (B) asthenozoospermic group, and (C) teratozoospermic group. Acc, accuracy; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity. (Color version available online.)

Table 2. Sperm parameters and ORP changes in all patients (N = 28) who had repeated tests done over a period of 16.8 ± 7.7 weeks

<table>
<thead>
<tr>
<th>Patients (N = 28)</th>
<th>First Sample</th>
<th>Second Sample</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (10⁶ sperm/mL)</td>
<td>7.2 (2.93, 20.73)</td>
<td>10.55 (5.22, 33.3)</td>
<td>.019</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>32 (17.5, 47.5)</td>
<td>42.5 (29.25, 53.75)</td>
<td>.008</td>
</tr>
<tr>
<td>Morphology (normal form, %)</td>
<td>2 (1.0, 5.5)</td>
<td>2 (1.25, 4.75)</td>
<td>.57</td>
</tr>
<tr>
<td>ORP (mV/10⁶ sperm)</td>
<td>6.08 (2.63, 15.92)</td>
<td>2.06 (0.7, 9.16)</td>
<td>.007</td>
</tr>
<tr>
<td>Patients treated with doxycycline (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endtz (M/mL)</td>
<td>0.8 (0.4, 2.3)</td>
<td>0 (0, 0.4)</td>
<td>.024</td>
</tr>
<tr>
<td>ORP (mV/10⁶ sperm)</td>
<td>0.77 (0.49, 1.05)</td>
<td>0.62 (0.49, 0.74)</td>
<td>.086</td>
</tr>
</tbody>
</table>

Abbreviation as in Table 1

Values are presented as median (25th and 75th percentiles).
OS-induced infertility. The role of ORP in monitoring relevant diseases and predicting pregnancy or assisted reproductive technology outcomes will require further studies.

References


APPENDIX

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.urology.2017.02.016.