Oxidation-Reduction Potential (ORP) as a Marker of Sperm Quality

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ABSTRACT

Objective: Oxidation-Reduction Potential (ORP) is an easy and effective measure of oxidative stress. A negative association between the poor semen parameters and oxidative stress has been reported. However, no study has evaluated the potential of ORP to serve as a surrogate marker of sperm quality. The objectives of our study were to evaluate if ORP has the potential to replace standard semen analysis as a surrogate marker of sperm quality.

Design: ORP measurement in infertile men.

Materials and Methods: Sperm parameters were evaluated in 301 infertile patients and 301 infertile men according to WHO 5th edition guideline. ORP was measured by MIOXSYS analyser. Sera were obtained by applying a cut-off was identified for which a notably higher percentage of patients were above the ORP cut-off than the donors. Sperm parameters were compared in infertile men with low ORP and high ORP.

Results: We identified a low cut-off of ≤1.30 mVolts/10^6 sperm and ≤5.14 mVolts/10^6 sperm, as high ORP cut-off. The relationship between sperm parameters and ORP is shown in Table 1. Significantly poor semen parameters were seen in patients with ORP >5.14 when compared to those with low ORP <5.14 (p <0.001). Donors had significantly better sperm quality at this cut-off.

Conclusions: We demonstrated a direct relationship between poor semen quality and high ORP which can serve not only as a reliable marker of oxidative stress but also has the potential to serve as a surrogate marker of sperm quality.

INTRODUCTION

Infertility is a global public health issue, affecting approximately 48.5 million couples or 12% of men globally. Most clinicians rely on the “gold standard” test, conventional semen analysis, as a surrogate measure of a man’s fertility ability. However, semen analysis has a poor predictive ability due to its highly technical (intra- and inter-assay) variability and the lack of stability of individual sperm parameters. Oxidative stress (OS) is a well-recognized key mediator in the pathogenesis of male infertility. OS leads to lipid peroxidation of sperm membranes and intracellular lipids and proteins, aggraves apoptosis, and results in DNA damage. Such effects are harmful to sperm cells, resulting in poor sperm quality and consequently reduced fertility. Conventional methods of OS measurements include chemiluminescent reactive oxygen species assay, total antioxidant capacity (TAC) test, ROS-TAC score and malondialdehyde (MDA) measurement. Although these methods provide useful information, these tests measure only a known or a discrete quantity of oxidants. They are mostly time-consuming and require large and expensive equipment as well as statistical modeling. Recently, novel technology measuring oxidation-reduction potential (ORP), the MIOXSYS analyser has been developed. ORP is a direct measurement of oxidative stress therefore the redox imbalance between oxidants and antioxidants in biological samples. 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. The objectives of our study were to evaluate (a) if ORP has the potential to replace standard semen analysis as a surrogate marker of sperm quality and (b) ORP cut-off values indicative of high ORP or oxidative stress.

MATERIALS AND METHODS

Semen samples were obtained from 301 infertile patients who attended the Reproductive Medicine Unit at Cleveland Clinic, Cleveland, OH, from August 2015 to August 2017. All patients were evaluated by a male infertility specialist. All infertile patients were seeking treatment for male infertility (idiopathic infertility, varicoceles, infection, and other known etiologies). Patients with infection, azoospermia, severe oligozoospermia (<1×10^6 sperm/mL) and retrograde ejaculation were not included in the study. The control group consisted of 84 normal healthy men of proven or unproven fertility with normal semen parameters for sperm concentration, motility and morphology according to WHO 5th edition guideline (WHO, 2010).

Semen analysis
Semen samples were collected in sterile containers after a period of 48-72h 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 (WHO, 2010). In addition, an Endtz-test to identify peroxidase-positive cells was performed. An aliquot of 5 µl of the sample was loaded on a MircCell counting chamber (Vitrolife, San Diego, CA, USA) 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, IL, USA) 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 cut-off.

Measurement of ORP
ORP was measured in millivolts (mV) using the galvanostat-based technology using the MIOXSYS system (Aytu Bioscience, Englewood, CO, USA). It consists of the MIOXSYS Analyzer and a sensor with the loading port and the reference port (Figure 1A-B). The sensor is inserted into the loading socket and 30 µL aliquot of liquefied semen was added on the loading port (Figure 2A-B). The static ORP was measured by applying a low voltage current and the whole process takes less than 4 minutes. The sensor is removed and ORP was normalized with the sperm concentration. The measured ORP reflects a “snapshot” of the current balance of the redox system and a higher ORP level suggests a higher state of OS.

Statistical Analysis
Data are presented as median (25th, 75th percentile). A p value of <0.05 was considered as statistically significant for pairwise groups comparison using Wilcoxon signed rank test and unpaired groups using Wilcoxon rank sum test. The correlation between the ORP and sperm parameters were analysed by Spearman’s rank order. The best cut-off value, sensitivity, specificity, positive predictive value, negative predictive value and area under the curve (AUC) were calculated by receiver operating characteristic (ROC) analysis. Box-and-Whisker plots were used to demonstrate the distributions of the ORP (mV/10^6 sperm) cut-off between control and patient group.

RESULTS

Distribution of ORP in control and patients is shown in Figure 3. Table 1.

1. The control group comprised of healthy men of proven or unproven fertility with normal semen parameters and did not present with leukocytospermia. The infertile group significantly presented with poor sperm concentration, total sperm concentration, motility and morphology.

2. An ORP reference value of 1.30 millivolts/10^6 sperm/ml was selected (Figure 1). In the infertile group 41.9% of the subjects had ORP <1.30 compared to 69 % in the control group.

3. In the control group, 94% of the subjects had an ORP<5.14 millivolts/10^6 sperm/ml compared to only 65.8% in the infertile group.

4. When the subjects were divided based on ORP into those with ORP <5.14 millivolts/10^6 sperm/ml versus >5.14 millivolts/10^6 sperm/ml only 6% of the subjects exhibited ORP >5.14 millivolts/10^6 sperm/ml compared to 34.2% of the patients.

5. Compared to the control group, in the patient group significantly poor semen parameters were seen with ORP >5.14 millivolts/10^6 sperm/ml (Table 1).

Figure 1. The MIOXSYS system consists of A: Analyzer and B: Sensor with sample port to load the sample.

Figure 2. Measurement of ORP showing A: Loading of sample into sample port and B-C. Insertion of sensor in the analyzer.

Figure 3. Box plots showing the distribution of ORP in controls (Donors) and Patients.

Table 1. Correlation of ORP with sperm concentration and motility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Donors (n = 84)</th>
<th>Patients (n = 301)</th>
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<tbody>
<tr>
<td>Sperm concentration (10^6)</td>
<td>52.85 ± 5.14</td>
<td>47.30 ± 13.64</td>
</tr>
<tr>
<td>Total sperm count (10^6)</td>
<td>131.55 ± 15.12</td>
<td>94.22 ± 32.57</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>56.6 ± 1.5</td>
<td>62.5 ± 6.1</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>6.9 ± 0.7</td>
<td>8.3 ± 1.8</td>
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Values are means ± SE, P<0.05 considered significant by Wilcoxon’s rank sum test.

CONCLUSION

1. We demonstrated a direct relationship between ORP and poor semen quality.
2. ORP values >5.14 millivolts/10^6 sperm/ mL can differentiate patients from controls. Values >5.14 millivolts/10^6 sperm/ mL are indicative of oxidative stress and indicative of poor sperm quality.
3. Combination of ORP with semen parameters may be more meaningful and ORP can serve as surrogate marker.
4. ORP may be effective in differentiating infertile men with different semen parameters and etiologies.
5. ORP may be potentially used in different clinical settings as a screening or confirmatory test. The use of ORP as a standardized OS measurement in future research facilitate the development of effective intervention for OS-induced infertility.