Validation of oxidation-reduction potential in fresh and frozen semen samples with MiOXSYS™ System

Ashok Agarwal, Rakesh Sharma, Stefan Du Plessis and Edmund Sabanegh

American Center for Reproductive Medicine and Department of Urology, Cleveland Clinic, Cleveland, OH

ABSTRACT

INTRODUCTION AND OBJECTIVES: Oxidative stress in seminal ejaculate is quantified by measuring individual markers such as reactive oxygen species, lipid peroxidation and total antioxidants. However, they have limited diagnostic capabilities. Oxidation-reduction potential (ORP) is a measure of oxidative stress using the MiOXSYS System. It provides a better measure of oxidative stress compared to individual markers.

METHODS: Sample semen was collected from healthy normospermic subjects after 2-3 days of abstinence. Oxidative stress was induced with cumene hydroperoxide (CH) at 5 and 50 µm. Oxidative stress was measured using a novel galvanostat-based technology, the MiOXSYS System. A 30 µl aliquot of the test sample was applied to the sensor. The Sensor is inserted into the Analyzer; measurement begins automatically and takes 3 to 4 minutes. After thawing the samples, ORP was measured again in all three fractions in triplicate using the method described above. The change in pre-freeze and post-thaw ORP was compared.

RESULTS: Both 5 and 50 µm C/106 sperm/mL for cORP. Change in pre-freeze and post-thaw sORP/106 sperm/mL for 5 µm CH: 0.28 ± 0.12 (-0.40, 0.05); for 50 µm CH: 0.12 ± 0.09 (-0.31, -0.06). Change in pre-freeze and post-thaw cORP/106 sperm/mL: for 5 µm CH: 0.28 ± 0.06 (-0.40, -0.16); for 50 µm CH: 0.31 ± 0.24 (-0.20, 0.81). A P-value of < 0.05 was considered statistically significant.

CONCLUSIONS: The MiOXSYS System can measure oxidative stress in both fresh and frozen semen specimens. It is a simple and real-time measure of oxidative potential that can be used by fertility laboratories.

INTRODUCTION

The imbalance between ROS production and scavenging by antioxidants causes oxidative stress, which deteriorates sperm quality, fertilizing ability, chances of natural pregnancy as well as outcomes of assisted reproductive techniques. Oxidative stress can be quantified by measuring the levels of ROS in the seminal ejaculate and total antioxidant capacity (TAC) in the seminal plasma. Earlier, we demonstrated that a combined ROS-TAC score provides a better measure of oxidative stress compared to evaluating individual ROS and TAC parameters at any given time. However, this often results in an unreliable, highly variable and conflicting evaluation of a patient's redox balance and also involves the use of sophisticated instruments, for example, luminometer, flow cytometer, plate reader, etc. Such tools are cumbersome, require large volume of seminal fluid, and have high turn-around-time for test results. In addition, (1) are unable to provide sufficient diagnostic value on their own, (2) do not provide a complete picture of redox imbalance as miss other important factors that contribute to a semen sample’s overall redox balance; (3) require multiple manual inputs, which lead to occasional errors; and (4) must be performed manually, time-consuming steps. Another major limitation is that accurate ROS levels and accurate antioxidant reserves in semen are often obtained with manual inputs, which can lead to complex errors; and must be performed in a lab setting with a luminometer, flow cytometer, plate reader, etc. Such tools are cumbersome, require large volume of seminal fluid, and have high turn-around-time for test results.

The dynamics of the MiOXSYS System in evaluating sORP and cORP and validates the effects of oxidative stress using the MiOXSYS System for 1) measurement ORP in semen before and after freezing of effect of exogenous induction of oxidative stress by cumene hydroperoxide (CH) on ORP.

MATERIAL AND METHODS

Study Samples and Semen Analysis

This study was reviewed and approved by the Institutional Review Board; semen samples were collected from healthy normospermic men (n = 20) after 2-3 days of sexual abstinence. Semen samples were analyzed according to the 2010 World Health Organization (WHO) guidelines. After complete liquefaction, a 5µl aliquot of each sample was loaded on a Microlit chamber counting vial (Gilson, San Diego, CA) and manually evaluated for sperm concentration (>10^6/mL), percentage motility, number of round cells per high power field by using 20x phase contrast objectives. Samples that were highly viscous or had > 100 round cells were excluded from the study.

Methods:

Sample processing and induction of oxidative stress by cumene hydroperoxide

Each sample was divided into three aliquots: 1) aliquot 1 - incubated with sperm and medium (control); aliquots 2 and 3 were exposed to oxidative stress by freshly prepared cumene hydroperoxide (CH 5 and 50µmoles) in PBS and incubated for 20 minutes at 37°C. CH : semen v/v. All aliquots were exposed to oxidative stress without any cryoprotectant for 24 hours for measurement of ORP if frozen samples.

Measurement of oxidation-reduction potential

Oxidative stress is defined as a measure of transfer of electrons from a reducer (or antioxidant) to an oxidant and was measured using a novel galvanostat-based technology, the MiOXSYS System. The change in cORP in higher was in the controls vs. the sperm treated with 50 µm (P<0.001) in both pre- and post-freeze semen samples.

RESULTS:

Effect of cumene hydroperoxide on sperm motility and seminal ORP

Changes in pre-thaw and post-thaw seminal sORP and cORP after exposure to cumene hydroperoxide

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>Change in pre-freeze and post-thaw sORP/106 sperm/mL</th>
<th>Change in pre-freeze and post-thaw cORP/106 sperm/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>sORP/106 sperm/mL</td>
<td>Control</td>
<td>0.32 ± 0.11 (-0.55, -0.09)</td>
<td>0.009</td>
<td>5µ moles of CH 0.28 ± 0.06 (-0.40, -0.16) &lt;0.001</td>
</tr>
<tr>
<td>sORP/106 sperm/mL</td>
<td>5µmoles of CH</td>
<td>0.31 ± 0.24 (-0.20, 0.81)</td>
<td>0.22</td>
<td>50µmoles of CH 0.26 ± 0.10 (-0.48, -0.05) &lt;0.02</td>
</tr>
<tr>
<td>cORP/106 sperm/mL</td>
<td>Control</td>
<td>1.33 ± 3.42 (0.98, 6.25)</td>
<td>0.02</td>
<td>5µ moles of CH 1.10 ± 0.29 0.49, 1.70 0.82 ± 0.27 0.26, 1.37 &lt;0.001</td>
</tr>
<tr>
<td>cORP/106 sperm/mL</td>
<td>5µmoles of CH</td>
<td>1.62 ± 3.84 (1.27, 4.96)</td>
<td>0.17</td>
<td>50µmoles of CH 1.34 ± 0.41 (0.95, 1.74) 0.04</td>
</tr>
</tbody>
</table>

Comparison of oxidation-reduction potential (ORP) following exposure to 5 and 50µmoles of cumene hydroperoxide (CH)

Effect of freezing on seminal sORP and cORP after exposure to cumene hydroperoxide

Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>Change in pre-freeze and post-thaw sORP/106 sperm/mL</th>
<th>Change in pre-freeze and post-thaw cORP/106 sperm/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>sORP/106 sperm/mL</td>
<td>Control</td>
<td>0.41 (95% CI: 1.43, 3.14)</td>
<td>0.76</td>
<td>5vs. 50µmoles of CH 0.00 ± 0.15 (-0.30, 0.30) &gt;0.99</td>
</tr>
<tr>
<td>sORP/106 sperm/mL</td>
<td>5µmoles of CH</td>
<td>0.49 ± 0.17 (0.26, 0.73)</td>
<td>0.08</td>
<td>5 vs. 50µmoles of CH 0.02± 0.10 (-0.20, 0.24) 0.86</td>
</tr>
<tr>
<td>sORP/106 sperm/mL</td>
<td>50µmoles of CH</td>
<td>0.52 ± 0.24 (0.03,0.10)</td>
<td>0.05</td>
<td>5 vs. 50µmoles of CH 0.00 ± 0.15 (-0.30, 0.30) &gt;0.99</td>
</tr>
</tbody>
</table>

Comparison of oxidation-reduction potential (ORP) following exposure to 5 and 50µmoles of cumene hydroperoxide (CH)

Effect of freezing on seminal sORP and cORP after exposure to cumene hydroperoxide

Figure 1. Comparison of change in pre-freeze and post-thaw seminal sORP and cORP after exposure to cumene hydroperoxide

Figure 2. Comparison of oxidation-reduction potential (ORP) following exposure to 5 and 50µmoles of cumene hydroperoxide (CH)

A: Pre-thaw ORP/106 sperm/mL; B: Pre-thaw ORP/106 sperm/mL; C: Post-thaw ORP/106 sperm/mL; D: Post-thaw ORP/106 sperm/mL; E: Change in pre-thaw and post-thaw ORP/106 sperm/mL; F: Change in pre-thaw and post-thaw ORP/106 sperm/mL.

The gray boxplots demonstrate the percentiles (5th, 25th, median, 75th, and 95th) and the red dots and lines show the mean and its 95% confidence interval.

CONCLUSIONS:

1. MiOXSYS System can measure the ORP in both fresh and frozen semen samples in a single test.

2. ORP technique is sensitive to changes in ORP following induction of oxidative stress by cumene hydroperoxide.

3. Induction of oxidative stress by CH, and measurement of ORP in real time supports the dynamics of the MiOXSYS System in evaluating sORP and cORP and validates the measurements in human semen.

4. Further studies utilizing larger sample size and in multicenter format using semen samples from infertile men are important to verify its clinical utility as an alternative to current oxidative stress markers. This will introduce an easy, simple, and real-time measure of oxidative stress and ORP in Andrology and IVF laboratories.

Figure 1. Measurement of oxidation-reduction potential (ORP) by Analyzer by placing the sensor; B: gently inserting the sensor; C: view when the sensor is in place.

Figure 2A. Validation of oxidation-reduction potential in fresh and frozen semen samples with MiOXSYS™ System.