Cumene hydroperoxide induced changes in oxidation–reduction potential in fresh and frozen seminal ejaculates

A. Agarwal1 | R. Sharma1 | R. Henkel2 | S. Roychoudhury1,3 | S. C. Sikka4 | S. du Plessis5 | Y. B. Sarda1 | C. Speyer1 | M. Nouh1 | C. Douglas1 | Z. Kayali1 | E. S. Ahmed1 | E. Sabanegh6

1American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA
2Department of Medical Bioscience, University of the Western Cape, Bellville, South Africa
3Department of Life Science and Bioinformatics, Assam University, Silchar, India
4Tulane University Health Sciences Center, New Orleans, LA, USA
5Medical Physiology, Stellenbosch University, Tygerberg, South Africa
6Department of Urology, Cleveland Clinic, Cleveland, OH, USA

Correspondence
Professor Ashok Agarwal, Lerner College of Medicine, Andrology Center and American Center for Reproductive Medicine, Cleveland, OH, USA.
Email: agarwaa@ccf.org

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American Center for Reproductive Medicine

Summary
Oxidation–reduction potential (ORP) is a newer integrated measure of the balance between total oxidants (reactive oxygen species—ROS) and reductants (antioxidants) that reflects oxidative stress in a biological system. This study measures ORP and evaluates the effect of exogenous induction of oxidative stress by cumene hydroperoxide (CH) on ORP in fresh and frozen semen using the MiOXSYS Analyzer. Semen samples from healthy donors (n = 20) were collected and evaluated for sperm parameters. All samples were then flash-frozen at −80°C. Oxidative stress was induced by CH (5 and 50 μmoles/ml). Static ORP (sORP—(mV/10⁶ sperm/ml) and capacity ORP (cORP—μC/10⁶ sperm/ml) were measured in all samples before and after freezing. All values are reported as mean ± SEM. Both 5 and 50 μmoles/ml of CH resulted in a significant decline in percent motility compared to control in pre-freeze semen samples. The increase in both pre-freeze and post-thaw semen samples for sORP was higher in the controls than with 50 μmoles/ml of CH. The change from pre-freeze to post-thaw cORP was comparable. The system is a simple, sensitive and portable tool to measure the seminal ORP and its dynamic impact on sperm parameters in both fresh and frozen semen specimens.

KEYWORDS
antioxidants, cumene hydroperoxide, oxidation–reduction potential, oxidative stress, seminal ejaculate, sperm

1 | INTRODUCTION

Reactive oxygen species (ROS) are necessary for normal physiological functions of the male gamete including processes, such as capacitation, hyperactivation and acrosome reaction (Agarwal et al., 2015; Aitken & Fisher, 1994; Sharma & Agarwal, 1996a). When ROS levels become too high, they initiate cellular redox changes resulting in tissue damage by modifying lipids, proteins and DNA (Pasqualotto et al., 2000; Saleh & Agarwal, 2002; Sikka, 2001). This imbalance between ROS production and scavenging by antioxidants causes oxidative stress, which deteriorates sperm quality, fertilising ability, chances of natural pregnancy and outcomes of assisted reproductive techniques (El-Taieb et al., 2009; Ko et al., 2014; Kumar et al., 2009; Pons-Rejraji et al., 2009; Zorn, Vidmar, & Meden-Vrtovec, 2003).

Oxidative stress can be quantified by measuring the levels of ROS in the semen and total antioxidant capacity (TAC) in the seminal plasma (Kashou et al., 2013; Mahfouz et al., 2009; Miller et al., 1993; Roychoudhury et al., 2016). In an earlier study, Sharma et al., (1999) 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, electron spin resonance (ESR) spectroscopy and plate reader. Such tools are cumbersome, require
large volume of fresh seminal fluid and have a high turnaround time for test results. In addition, these techniques (i) are unable to provide sufficient diagnostic value on their own; (ii) do not provide a complete picture of the redox imbalance as they miss other important factors that contribute to a semen sample’s overall redox balance; (iii) require multiple manual inputs, which can lead to complex errors; and (iv) must be performed in multiple, time-consuming steps. Another major limitation is that accurate ROS levels cannot be measured in frozen semen samples by the chemiluminescence or ESR spectroscopy methods.

We have recently evaluated another measure of oxidative stress based upon oxidation–reduction potential (ORP), which is an integrated quantitative measure of the balance between total oxidants (ROS) and total reductants (antioxidants) in a biological system (Agarwal et al., 2016). It provides an overall picture of the general redox status as result of the total activity of oxidants and antioxidants causing redox imbalance and has been shown to correlate with the ability to respond to related illness or injury (Bar-Or et al., 2005; Stagos et al., 2015). Static ORP (sORP) and capacitance ORP (cORP) are two integral measures of the redox system that have been reported to correlate well with important clinical features in critical injuries and illnesses such as isolated traumatic brain injury, liver disease, multitrauma injury and stroke (Bar-Or et al., 2009; Rael et al., 2009a, 2009c). Static ORP is a “snapshot” of the current redox balance; a higher sORP reading is indicative of oxidative stress. Capacity ORP reflects the amount of antioxidant reserves available in a given system. Their role in male infertility is not yet known, and there are no reports on the utility of using ORP as an alternative to measuring oxidative stress in the seminal ejaculate.

The goals of the current study were to evaluate the feasibility of using these two parameters, sORP and cORP, to measure the seminal redox status in human ejaculates before and after freezing, and examine the effect of exogenous induction of oxidative stress by cumene hydroperoxide (CH) on ORP.

2 | MATERIAL AND METHODS

2.1 | Study samples and semen analysis

This study was reviewed and approved by the Institutional Review Board of Cleveland Clinic, and all the human subjects included in the study had given written, informed consent for using their discarded semen samples for inclusion in the study. Fresh semen samples were collected from healthy normal men (n = 20; 18–40 years of age) after 2–3 days of sexual abstinence. These men were of proven fertility and presented with normal semen parameters according to the WHO guidelines (World Health Organization, 2010).

Semen samples were analysed according to the 2010 World Health Organization (World Health Organization, 2010) criteria. After complete liquefaction, a 5 μl aliquot of a well-mixed sample was loaded on a MicroCell counting chamber (Vitrolife, San Diego, CA, USA) and manually evaluated for sperm concentration (×10⁶/ml), percentage motility, number of round cells per high power field using 20x phase contrast optics. Samples that were highly viscous or had >1 × 10⁶/ml round cells were excluded from the study.

2.2 | Sample processing and induction of lipid peroxidation by cumene hydroperoxide

Each sample was divided into three aliquots: aliquot 1 was incubated with sperm wash medium (Sage BioPharma, Bedminster, NJ, USA) that served as control. To induce oxidative stress, aliquots 2 and 3 were exposed to freshly prepared cumene hydroperoxide (CH) (5 and 50 μmoles/ml, technical grade 80%, Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (Hughes et al., 2010) and incubated for 20 min at 37°C. All fractions were snap-frozen (−80°C) without any cryoprotectant for 24 hr for the measurement of ORP in frozen samples.

2.3 | Measurement of the oxidation–reduction potential (ORP)

Oxidation–reduction potential is a measure of the transfer of electrons from a reductant (or antioxidant) to an oxidant and was measured using a novel galvanostat-based technology—the MiOXSYS system comprised of analyser and the sensor (Aytu Bioscience, Englewood, CO, USA). The MiOXSYS Analyzer provides two measures of oxidative stress. The static ORP (sORP) (measured in mV) is the integrated measure of the existing balance between total oxidants and reductants in a biological system. Immediately after the initial sORP reading is recorded, the analyser automatically applies a small current sweep to the sample, resulting in the exhaustion of all the antioxidant species, providing a measure of antioxidant capacity reserve called as capacitance ORP (cORP; measured in micro Coulombs—μC). Calibration of the MiOXSYS Analyzer and subsequent measurements were carried out according to the manufacturer’s instructions. After complete liquefaction, semen analysis was carried out and ORP measurements were conducted immediately. Testing began automatically when the sample filled the reference electrode, thereby completing the electrochemical circuit, and took 3–4 min.

For measuring ORP after freezing, the samples were placed in the incubator at 37°C for 20 min. After the samples were completely thawed, ORP was measured in all three fractions in triplicate. The average values for sORP and cORP were recorded. Data were normalised to sperm concentration in order to account for differences in cell numbers and were presented as mV/10⁶ sperm/ml for sORP and μC/10⁶ sperm/ml for cORP.

2.4 | Statistical analysis

Summaries of the categorical variables are reported as per cent frequency, quantitative variables as mean ± SEM. Data were tested for normal distribution by means of the Kolmogorov–Smirnov test. According to normal distribution, parametric or nonparametric tests were employed. Paired t-test was used for measuring per cent change in sORP and cORP in pre-freeze and post-thaw samples, while the Wilcoxon signed-rank test for pairwise group comparison was used to compare the significant difference between control and two doses of CH. Trends were analysed according to the normal distribution of
the data using either the Kruskal–Wallis test or one-way ANOVA. A p-value of $p < .05$ was considered significant for comparisons of each of the two treatment groups to controls, applying a Bonferroni correction.

3 | RESULTS

Descriptive summary statistics of the semen parameters are provided in Table 1.

### 3.1 Effect of cumene hydroperoxide (CH) on sperm motility

Exposure of washed sperm to oxidative stress by cumene hydroperoxide caused a significant ($p < .0001$) dose-dependent decrease in human sperm motility; the values decreased from initial 52.18 ± 2.31% (control) by 20.6% to 41.45 ± 2.09% (5 μmoles/ml CH). The highest concentration of CH (50 μmoles/ml) caused further reduction by 18.0% to 33.99 ± 2.23%.

### 3.2 Effect of cumene hydroperoxide on sperm viability and ORP before freezing

Exposure to 5 and 50 μmoles/ml of CH resulted in a significant, dose-dependent decline in pre-freeze viability ($p = .0072$) compared to the control (60.91 ± 2.29%; Table 2). The total per cent decrease from the control to the highest concentration of CH used was 21.4%.

CH increased the available oxidants resulting in an increase in sORP (mV/10⁶/ml sperm) before freezing (Table 2). Yet, despite significant differences (Table 3) in the control and 5 and 50 μmoles/ml CH, respectively, no trend ($p = .8262$) could be established. Conversely, the cORP (μC/10⁶/ml) levels showed a decline in the available antioxidant reserves following exposure to both 5 and 50 μmoles/ml of CH (Table 2) with significant differences between the control and 5 and 50 μmoles/ml CH (Table 3), respectively; however, no trend ($p = .5959$) was seen.

### 3.3 Effect of cumene hydroperoxide on sperm viability and ORP post-freezing

For sperm viability after post-freezing, the same trend was seen with a significant ($p = .0002$) dose-dependent decline of sperm viability as before freezing. Although values for sORP increased from 2.29 to 3.03 mV/10⁶/ml and for cORP decreased from 0.96 to 0.83 μC/10⁶/ml, these trends were not significant ($p = .6294$ and $p = .7584$ respectively) (Table 2), despite the fact that some differences between different CH concentrations were significant (Table 3).

The individual changes observed for the effect of oxidative stress induced by cumene hydroperoxide on the different parameters as well as of the freezing process itself are shown in Figure 1.

### 3.4 Effect of freezing on sperm viability, sORP and cORP under the influence of cumene hydroperoxide

A significant ($p < .0001$) decrease in sperm viability was seen after freezing (Table 2), which was significantly amplified under the influence of oxidative stress; 50 μmoles/ml of CH caused a 21.4% decline in the pre-freeze samples, while the same concentration caused a 64.9% decline post-freeze ($p < .0001$).

For sORP, the changes affected by oxidative stress from the control to the highest concentration of CH used in the pre-freeze and post-freeze samples were 10.7% and 44.3% respectively, while the

### Table 1

Summary statistics of standard semen parameters before freezing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.52±0.22</td>
</tr>
<tr>
<td>pH</td>
<td>7.67±0.05</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>4.67±0.67</td>
</tr>
<tr>
<td>Sperm count (×10⁹/ml)</td>
<td>33.87±5.20</td>
</tr>
<tr>
<td>Total sperm count (×10⁶)</td>
<td>118.83±20.15</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>52.18±2.31</td>
</tr>
</tbody>
</table>

### Table 2

Summary statistics of sperm viability and seminal static oxidation-reduction potential (sORP) and capacitance oxidation-reduction potential (cORP) after exposure to cumene hydroperoxide (CH) before and after freezing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment with CH</th>
<th>Pre-freeze (mean ± SEM)</th>
<th>Post-thaw (mean ± SEM)</th>
<th>p-value</th>
<th>Kruskal–Wallis (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (%)</td>
<td>Control</td>
<td>60.91±2.29</td>
<td>5.69±1.57</td>
<td>&lt;.0001</td>
<td>&lt;.0072</td>
</tr>
<tr>
<td></td>
<td>5 μmoles/ml</td>
<td>53.93±2.40</td>
<td>3.85±0.70</td>
<td>&lt;.0001</td>
<td>.0002</td>
</tr>
<tr>
<td></td>
<td>50 μmoles/ml</td>
<td>47.73±2.49</td>
<td>1.57±0.22</td>
<td>.8262</td>
<td>.8262</td>
</tr>
<tr>
<td>sORP (mV/10⁶ sperm)</td>
<td>Control</td>
<td>2.21±0.40</td>
<td>2.29±0.41</td>
<td>.3011</td>
<td>.8262</td>
</tr>
<tr>
<td></td>
<td>5 μmoles/ml</td>
<td>2.49±0.48</td>
<td>2.68±0.50</td>
<td>.1443</td>
<td>.6294</td>
</tr>
<tr>
<td></td>
<td>50 μmoles/ml</td>
<td>2.73±0.54</td>
<td>3.03±0.60</td>
<td>.1070</td>
<td>.7584</td>
</tr>
<tr>
<td>cORP (μC/10⁶ sperm)</td>
<td>Control</td>
<td>1.27±0.32</td>
<td>0.96±0.31</td>
<td>.0002</td>
<td>.5959</td>
</tr>
<tr>
<td></td>
<td>5 μmoles/ml</td>
<td>1.10±0.29</td>
<td>0.82±0.27</td>
<td>&lt;.0001</td>
<td>.7584</td>
</tr>
<tr>
<td></td>
<td>50 μmoles/ml</td>
<td>1.10±0.36</td>
<td>0.83±0.31</td>
<td>.0028</td>
<td>.7584</td>
</tr>
</tbody>
</table>
While redox imbalance has been widely studied in multiple research settings, it has not been possible to accurately, easily and completely assess a sample’s overall redox status in real time. Individual surrogate markers have been historically used to measure redox imbalance in patients such as measuring antioxidants like glutathione, LPO, free radical production, protein oxidation and/or enzyme activity (Bar-Or et al., 2009; Rael et al., 2009b). Our study aimed to establish a platform to use the MiOXSYS system as a viable option to measure the ORP in human ejaculates and establish its utility as an easy, reliable, quick tool in clinical settings.

Biological importance and power of such instrument have been validated previously in human models (Bar-Or et al., 2005, 2009; Rael et al., 2009a, 2009b, 2009c; Stagos et al., 2015). Using the MiOXSYS Analyzer, sORP and cORP were measured in human blood in strenuous exercise-induced oxidative stress in athletes as well as in human semen and seminal plasma (Agarwal et al., 2016; Stagos et al., 2015).

We studied the effect of exogenous induction of oxidative stress on ORP. The induction of oxidative stress by CH as measured by MiOXSYS resulted in an increase in sORP both in the fresh and frozen samples from the normozoospermic men. CH has been used to assess the effects of ROS in biological models. Unlike hydrogen peroxide, this compound is relatively stable and slightly water soluble (Ayala et al., 1996; Fagali & Catala, 2007). CH has been shown to cause a dose-dependent decrease in both sperm motility and blastocyst development (Hughes et al., 2010).

This was a pilot study, and all samples were obtained from healthy men with normal semen parameters according to the World Health Organization, 2010 criteria. These men were not evaluated for infertility. It will be interesting to see in future studies how they compare between healthy men with proven and unproven fertility.

In this study, sORP levels increased following exposure to CH in fresh and frozen samples. Although the change in sORP between the controls and samples treated with 50 μmoles/ml of CH was significant in the direct comparison in both the pre-freeze and post-thaw samples, a significant trend could not be established. A similar observation was seen for cORP (Table 2). CH is an oxidant and known inducer of oxidative stress that has been used to assess the effect of free radicals and reactive oxygen species in various biological fluids. However, unlike hydrogen peroxide, CH is relatively stable, slightly water soluble and lipid soluble. CH concentrations ranging from 2.5 to 57.4 μM were tested by Hughes et al. (2010) using two-cell mouse embryos for blastocyst formation, rate of apoptosis in developing blastocysts by TUNEL assay and for both per cent motility.

**TABLE 3** Effect of cumene hydroperoxide (CH) on seminal sORP and cORP before and after freezing. The relevant average values are depicted in Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-freeze changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility (%)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles of CH</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.0012</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles of CH</td>
<td>.0009</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0001</td>
</tr>
<tr>
<td>sORP (mV/10⁶ sperm/ml)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.0217</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles of CH</td>
<td>.1137</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0047</td>
</tr>
<tr>
<td>cORP (μC/10⁶ sperm/ml)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.0204</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles/ml of CH</td>
<td>.3304</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0057</td>
</tr>
<tr>
<td><strong>Post-thaw changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viability (%)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.0742</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles/ml of CH</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0001</td>
</tr>
<tr>
<td>sORP (mV/10⁶ sperm/ml)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.0386</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles/ml of CH</td>
<td>.0883</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0055</td>
</tr>
<tr>
<td>cORP (μC/10⁶ sperm/ml)</td>
<td>Control versus 5 μmoles/ml of CH</td>
<td>.1154</td>
</tr>
<tr>
<td></td>
<td>5 versus 50 μmoles/ml of CH</td>
<td>.1134</td>
</tr>
<tr>
<td></td>
<td>Control versus 50 μmoles/ml of CH</td>
<td>.0575</td>
</tr>
</tbody>
</table>

The difference between these two values was significant (p = .0081). Although changes (28.9% in the pre-freeze versus 25.6% in the post-freeze) could be observed, this effect in the percent change between pre-freeze and post-freeze was not significant (p = .4852).

### 4 | DISCUSSION

While redox imbalance has been widely studied in multiple research settings, it has not been possible to accurately, easily and completely assess a sample’s overall redox status in real time. Individual surrogate markers have been historically used to measure redox imbalance in patients such as measuring antioxidants like glutathione, LPO, free radical production, protein oxidation and/or enzyme activity (Bar-Or et al., 2009; Rael et al., 2009b). Our study aimed to establish a platform to use the MiOXSYS system as a viable option to measure the ORP in human ejaculates and establish its utility as an easy, reliable, quick tool in clinical settings.

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and forward progression using the human sperm motility assay and sperm motility index as described by Claassens et al., (2000). At these concentrations, CH did not have any negative impact on the motility. Therefore, we selected two concentrations of CH (5 and 50 μM) for our experiment to measure ORP in fresh and frozen semen samples.

Cryopreservation is known to significantly impair motility, viability and morphology, and also causes oxidative stress-induced cryoinjury (Han et al., 2014; Karimfar et al., 2015; O’Connell et al., 2002; Sharma & Agarwal, 1996b; Zrbi et al., 2010) by increasing ROS production, DNA damage and apoptosis, and reducing antioxidant levels (Kalthur et al.,

**FIGURE 1** Comparison of oxidation–reduction potential (ORP) following exposure to 5 and 50 μmoles of cumene hydroperoxide (CH), apart from control. (a) Pre-freeze sORP/10^6 sperm/ml increased significantly (p = .04) after exposure to 50 μmoles/ml of CH compared to control; (b) pre-freeze cORP/10^6 sperm/ml did not change significantly; (c) post-thaw sORP/10^6 sperm/ml increased significantly (p = .025) after exposure to 50 μmoles/ml of CH compared to control; (d) post-thaw cORP/10^6 sperm/ml did not change significantly; (e) change in pre-freeze and post-thaw sORP/10^6 sperm/ml was not significant; and (f) change in pre-freeze and post-thaw cORP/10^6 sperm/ml in control as well as samples exposed to both 5 and 50 μmoles/ml of CH was significant. The grey boxplots demonstrate the percentiles (5th, 25th, median, 75th and 95th) of the data distributions at each concentration of CH. The blue lines show the changes for individual subjects between CH concentrations.
The assay is novel in the area of infertility where oxidative stress plays a significant role in inducing sperm damage and male infertility. The changes in ORP as demonstrated in our present study may be more significant and relevant when measured in infertile men with different clinical aetiologies. This is a limitation of the present study as we did not test the ability of CH on ORP in infertile patient samples. These changes in ORP may be more significant and relevant when correlated with abnormal semen parameters from infertile men with various clinical aetiologies. Nevertheless, the efficiency of the MiOXSYS system needs further testing in infertile men particularly with clinical diagnosis such as varicocele attributed to oxidative stress. However, it is important to note that MiOXSYS system can accurately measure ORP in fresh and frozen semen samples. It is recommended that for measurement of DNA fragmentation, the samples be shipped overnight on dry ice. Therefore, our objective was to measure the changes in ORP in fresh and frozen samples.

In a clinical setting, samples are cryopreserved with a cryoprotectant, usually test yolk buffer using the slow freezing technique. We also measured ORP in cryopreserved semen samples to study whether there are alterations in ORP in fresh and frozen samples after the samples are mixed with the cryoprotectant before freezing. We have demonstrated that ORP is a stable marker of oxidative stress (unpublished study). Measuring ORP in a given semen sample can also be beneficial in identifying the samples that are more likely to benefit from various treatment strategies, for example, from antioxidant therapy whether in vivo or during IVF cycles. Another limitation of the present study is that we did not simultaneously compare the ORP results of the MiOXSYS Analyzer with ROS and TAC measurements in the same seminal fluid. Measurements of ROS and TAC in semen samples have been performed in several previous studies (Agarwal et al., 2015; Roychoudhury et al., 2016; Sharma et al., 1999). Currently, the available methods of measuring oxidative stress parameters are tedious, time-consuming and need fresh ejaculates usually within few minutes of collection and liquefaction. These methods and instruments are not commonly available in any physician’s office or laboratory. Comparatively, the MiOXSYS system is a very small instrument, uses a sensor, requires very small amount of fresh or frozen semen sample (~30 μl), the results are available within minutes (3–4 min), and the equipment is portable and occupies minimum space. As the WHO has also acknowledged oxidative stress as an important parameter that plays a significant role in both male and female infertility, its assessment and management are critical for such patient care, especially in cases of idiopathic infertility. It provides an integrated measure of the balance between total known and unknown reductants and antioxidants in a biological system (Agarwal et al., 2016; Bar-Or et al., 2009; Rael et al., 2009b).

In conclusion, we have demonstrated that this technique can easily and accurately measure the ORP in small amounts of both fresh and frozen semen samples in real time. Furthermore, such measurements are sensitive to the changes in the ORP following exposure to oxidative stress-causing agent (CH). It also overcomes the challenge of measuring oxidative stress in frozen samples. Induction of oxidative stress by CH and measurement of ORP in real time support the dynamics of the MiOXSYS system in evaluating sORP and cORP, and validate the measurements in human semen. Further studies utilising 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 so as to establish this as an easy, simple and real-time measure of oxidative stress and ORP in Andrology and IVF laboratories.

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CONFLICT OF INTEREST

None of the authors declare competing financial interests.

REFERENCES


