# Seminal Oxidation-Reduction Potential

# 30

Mohit Rana and Ashok Agarwal

**Key Points**

- Oxidative stress (OS) has extensive implication on male infertility due to its ability to induce lipid peroxidation of the spermatozoa's membrane.
- Previous methodologies have been developed to assess how reactive oxygen species affect sperm directly or indirectly by decreasing total antioxidant capacity (TAC). However, these methods are time-consuming and technically demanding and require expensive instruments. Nitroblue tetrazolium (NBT) was an assay to avoid these pitfalls, but the uncertainty of what causes it to indicate OS calls into question its diagnostic accuracy.
- The MiOXSYS system was developed to allow an easy determination of oxidation-reduction potential without requiring an expensive instrument and laborious methodologies.
- Preliminary tests validated the MiOXSYS system's sensitivity to OS, its reliability, and reproducibility.
- The most accurate way to determine an oxidation-reduction potential cutoff is to assess semen with abnormality versus semen without abnormality. Several investigations have led to the refinement of the current cutoff of 1.34 mV/$10^6$ sperm/mL.
- Global studies on this cutoff value show a deviation cutoff ORP, suggests further investigations into how ORP and OS changes reflect ethnic-specific pathology.

## 30.1 Introduction

Currently, male infertility is assessed globally by performing a routine semen analysis, which is the cornerstone of andrology. However, it has received criticism in its ability to assess male infertility [1]. The controversies surrounding basic semen analysis stem from three discrepancies. The first is the failure of the World Health Organization (WHO) fifth edition semen analysis results to predict male fertility [2–5]. The second is the lack of inclusion of Middle East, Latin American, Asian, and African countries in the formation of the WHO fifth edition guidelines [6]. The exclusion of Northern and sub-Saharan African countries is particularly negligent as these countries have the highest global prevalence of infertility [7]. The third is the failure to assess the functional health of the sperm as semen analysis does not necessarily reflect the sperm's ability to fertilize the oocyte [5, 8].

Male fertility functional tests can provide a variety of information regarding the health of the sperm. Acrosome reaction testing can indicate if spermatozoa are able to undergo the necessary acrosome reaction to fertilize the oocyte [9]. Once sperm have prematurely undergone acrosome reaction, they cannot fertilize the oocyte, which indicates a pathological process in the semen [10–12]. Zona sperm penetration assay assesses if spermatozoa are capable of penetrating the zona pellucida membrane of the oocyte [13]. Inability of the spermatozoa to penetrate the zona pellucida indicates a pathological process either in capacitation, acrosome reaction, or zona pellucida binding/penetration [13, 14]. As reactive oxygen species (ROS) initiates acrosome reaction and facilitates zone pellucida penetration, an overproduction of ROS could cause premature initiation of either process and facilitate infertility, and subsequent functional tests could determine the etiology of infertility [9, 15, 16]. However, with the advent of intracellular sperm injection (ICSI), both functional tests provide little clinically useful information as ICSI is the resolution to both aforementioned pathologies [8]. Since ICSI bypasses

M. Rana · A. Agarwal (✉)
American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA
e-mail: mohit@mrana.net; agarwaa@ccf.org



S. J. Parekattil et al. (eds.), *Male Infertility*, https://doi.org/10.1007/978-3-030-32300-4_30

all biological barriers preventing sperm, an emphasis has been placed on sperm DNA fragmentation (SDF) as one of the last sperm factors that can influence fertilization success. One pathology which directly increases SDF is oxidative stress (OS).

## 30.2 Oxidative Stress

OS is the condition when ROS overwhelms spermatic antioxidants [17–20]. Sperm production of ROS was first investigated in 1943 when sperm incubated in high oxygen concentrations had improved motility with the addition of catalase [21]. While elevated ROS production decreases sperm viability, a small amount of ROS is needed to induce physiological processes of capacitation and acrosome reaction [22–24]. Therefore, ROS have both a beneficial and detrimental role in sperm function (Fig. 30.1).

Reactive oxygen species traditionally are free radical oxygen molecules with an unpaired electron but can also act as powerful oxidizers [25]. Hydrogen peroxide is an example of a powerful oxidizer and is significant in male infertility as it is more stable than other forms of ROS [26]. Free radical ROS are subdivided into categories based on the oxygen functional group that contains the radical [22, 23]. More clinically relevant subtypes of free radicals with regard to male infertility are peroxyl ($ROO^-$), hydroxyl (OH), and superoxide ($\cdot O_2^-$) [27, 28]. While ROS technically only have oxygen as the reactive atom, it has become an umbrella term to include other nonoxygen-based radicals too. Reactive nitrogen species (RNS) are involved in OS [29]. RNS subdivided into groups based on the functional nitrogen group

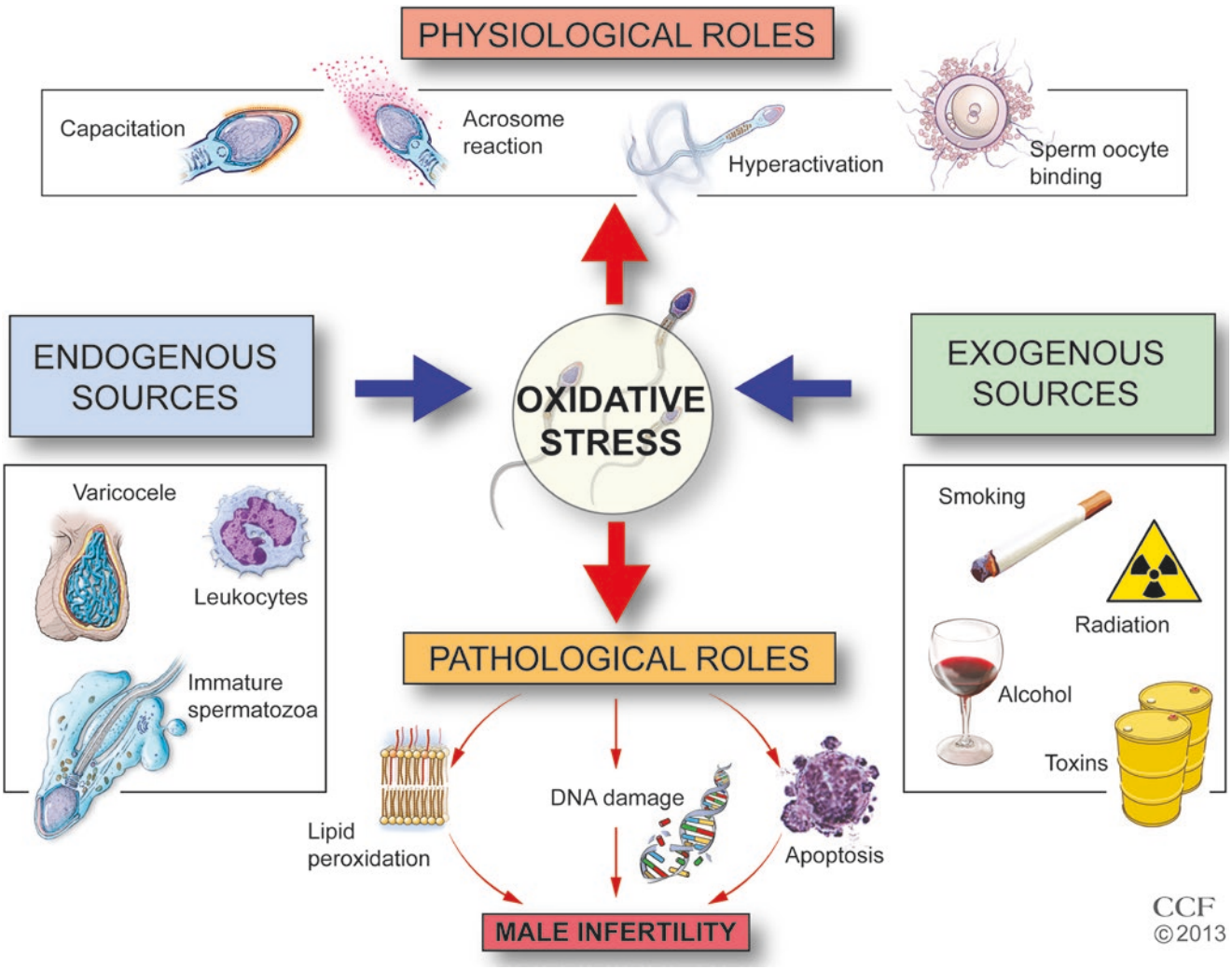


**Fig. 30.1** An overview of how reactive oxygen species are involved in physiological processes, such as capacitation and acrosome reaction. When ROS, from either endogenous or exogenous etiologies, are overabundant, pathological processes develop, thus leading to male infertility. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013–2018. All Rights Reserved)

attached to the radical, too [22]. Clinically relevant RNS are nitric oxide (NO) which, in reaction to superoxide, produces peroxynitrite ($ONOO^-$) [28, 29, 30].

Elevated reactive oxygen species are present in 20–80% of all male infertility patients [31], and antioxidant concentrations are depleted compared to fertile men [17, 18]. Sources of ROS can be either exogenous or endogenous to the spermatozoa. Endogenous sources of ROS are varicocele, diabetes mellitus type 2, metabolic syndrome, infection, immature spermatozoa, and elevated body temperature [26, 32–35]. Exogenous sources of ROS are alcohol abuse, drug abuse, mobile phone use, radiation, heavy metal exposure, prolonged exposure to high temperatures, and semen processing [36–40].

Spermatozoa can metabolically form superoxide, hydroxyl, and nitric oxide radicals [41]. In fertile men, metabolic ROS production is sequestered by enzymatic and low molecular antioxidants [42]. However, in idiopathic infertility, OS causes depletion of antioxidants and allows propagation of ROS-mediated damage [43, 44]. As spermiogenesis removes excess cytoplasm, deregulation of this process produces immature spermatozoa which retain an abundance of cytoplasm [17, 45]. The cytoplasm contains NADPH oxidase which continually produces ROS [46]. Leukocytospermia is a condition where leukocytes, particularly granulocytes, are excessively present ($>1 \times 10^6$/mL) in semen, which results in significant increase in ROS production [47, 48]. Leukocytes are particularly destructive as they produce up to 100 times the ROS compared to that of immature spermatozoa [49]. Sperm are susceptible to ROS-mediated damage by a variety of pathways. The first is the composition of the sperm plasma membrane primarily being comprised of polyunsaturated fatty acids which undergo electrophilic reactions with ROS, thus resulting in lipid peroxidation [18, 50–52]. Reactive oxygen species can destabilize the mitochondrial membrane potential which results in increased ROS production and depletion of adenosine triphosphate [53–56]. The most significant effect of ROS-mediated infertility is the increase in sperm DNA damage. In the presence of ROS, spermatic DNA forms base adducts which results in single-strand DNA breaks as spermatozoa lack the enzymes to repair a basic site (Fig. 30.2 [57–59]). High levels of SDF results in the failure of sperm to fertilize the oocyte in natural conception and assisted reproductive techniques [60–63]. Therefore, assays to determine ROS levels and their effects on antioxidant concentrations have been developed to understand and diagnose ROS-mediated infertility.

Assays to determine ROS in infertile men are evaluated by chemiluminescence, total antioxidant capacity (TAC), and, more recently, nitroblue tetrazolium (NBT) [31, 64–69]. Each methodology showed different pathologies with male infertility and presented with their own advantages and disadvantages (reviewed in Table 30.1). Chemiluminescence utilizes luminol as an ROS probe to determine concentrations of ROS either intracellular or extracellular to spermatozoa [70]. Elevated ROS results in lipid peroxidation of the sperm membrane, acrosome-reacted sperm, axoneme damage, and DNA damage [18, 50, 57, 71, 72]. TAC assays determine how well a semen sample can suppress the effects of ROS. Assays for the measurement of TAC utilize a source of ROS propagation and a probe which is sensitive to ROS-induced photon emission. The probe utilized can be either chemiluminescent, such

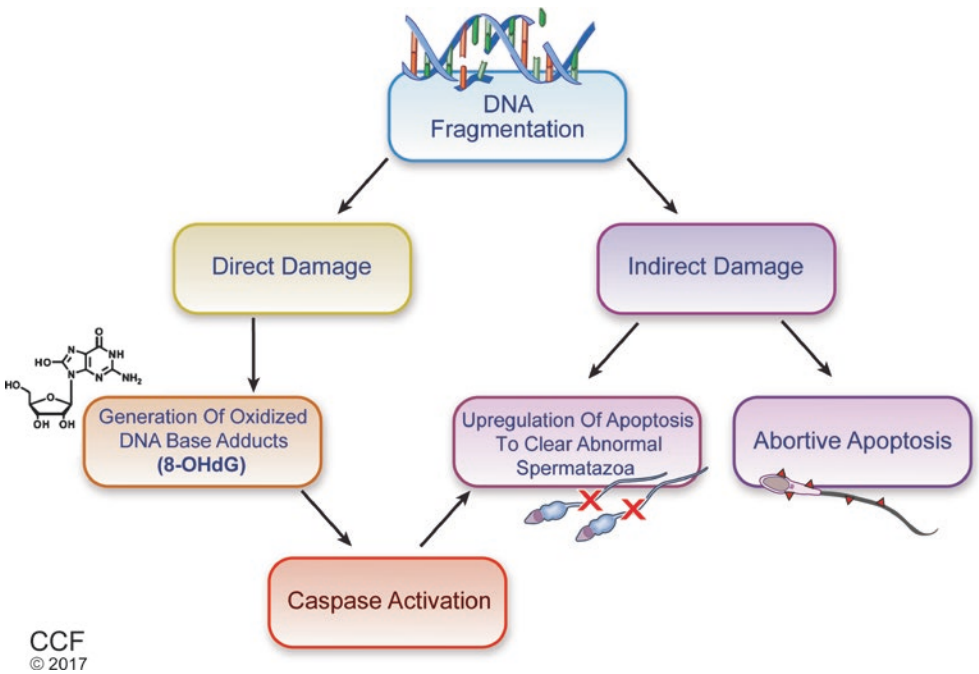


**Fig. 30.2** OS can directly lead to formation of 8-OHdG base adducts. As spermatozoa are unable to repair these adducts, spermatozoa shall enter apoptosis when fragmentation is elevated. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013–2018. All Rights Reserved)

**Table 30.1** Current assays used to determine OS in semen

| Assay | Description of finding | Advantages | Disadvantages | References |
|---|---|---|---|---|
| Chemiluminescence (ROS) | Commonly utilizes lucigen or luminol probes which react with ROS and emit a photon | The assay directly measures both intracellular and extracellular concentrations of ROS | Time-consuming, equipment is expensive, semen age affects the results, must be conducted in the dark to avoid interference with external light sources | [31, 64, 70] |
| Total antioxidant capacity (TAC) | The semen's ability to suppress chemiluminescence or colorimetric expression when exposed to a source of ROS determines the relative amount of antioxidant in the sample | Measures antioxidant concentration in semen Colorimetric method allows for a quick result | Requires expensive equipment; the length of the assay can change the results obtained | [66, 67, 121, 122] |
| ROS-TAC score | A statistical model used to determine semen quality and ability to compensate for OS | ROS-TAC scores are easy to comprehend, wholesome indicators of OS in semen | Time-consuming, requires calibration for the center's demographics prior to diagnostic capability, requires both a plate reader and a spectrophotometer | [19, 43, 123] |
| Nitroblue tetrazolium (NBT) | NBT is a clear, yellowish probe which turns indigo when exposed to ROS | Requires a fluorescent microscope, significantly reduces testing costs | NBT has questionable specificity for only ROS to cause the color changes | [69, 76, 124] |
| Thiobarbituric acid reactive substances (TBARS) | Able to detect lipid peroxidation by detecting MDA adduct formation | MDA formation is stable and is additive over time, showing OS damage over time Flow cytometer allows determination of 5000 sperm over the 200 from microscope evaluations | Requires a flow cytometer and strict quality control to produce reliable results | [125, 126] |
| Oxidation-reduction potential (ORP) | Provides a measurement of redox balance or ORP through an electrochemical measurement of all known and unknown oxidants and antioxidants | The only assay that measures redox balance exclusively. System procedure requires very little technician involvement and is easy to standardize | Affected by high levels of semen viscosity. Cannot be tested in stored samples containing sperm preparation media, buffer, or cryoprotectants | [77, 83, 112] |

as luminol, or colorimetric, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [66, 73]. The antioxidants in a semen sample will prevent either a photon emission or a color shift and, thus, produce the TAC. Low TAC values indicate a decrease in antioxidants present within the semen and subsequent pathological processes.

While chemiluminescence and TAC methodologies have advanced the pathological understanding of OS, they have poor diagnostic characteristics. Both methodologies are time-consuming, laborious, and require skilled personnel and expensive instruments [74, 75]. To circumvent these limitations, NBT was explored as an alternative to chemiluminescent techniques. To assess OS with NBT, a bright field microscope is required and visual acuity to determine sperm that have minimum color from those with dark-blue staining [68, 69]. While NBT has questionable specificity for oxidation strictly by ROS, the low cost of the assay allows NBT to be economical for developing nations, thus allowing OS to be standardized across the globe [46, 76]. Given the clinical significance of OS in male infertility evaluation, there is a need for a simple, cost-effective, and robust methodology.

## 30.3 The MiOXSYS System

The MiOXSYS system (Fig. 30.3) is an innovative system for diagnosing OS-induced infertility. The principle is to determine oxidation-reduction potential ($E^{ORP}$) of the Nernst equation (Eq. 30.1) [43, 43, 44]. The MiOXSYS system measures $E^{ORP}$ utilizing a galvanostatic measurement of a working solution [74, 77]. Galvanostatic measurements are not innovative in their own right, as they were originally described in 1941 [78]. The original use of these systems was to measure potential changes in electrolytic processes. The most widespread application of electrolytic chemistry was in municipal water supply chlorination [79–81]. Biological application of oxidation-reduction potential was observed in donor-acquired organs for transplantation, as ORP can be used to monitor ischemia-induced organ injury [82]. However, there was a limited use for ORP in biological samples due to the large volume of specimen required, the large size of the analyzers, and the impracticality of handling multiple samples in a timely manner [74, 80]. The MiOXSYS System enables the scope of ORP to be applied to semen and other biological samples:

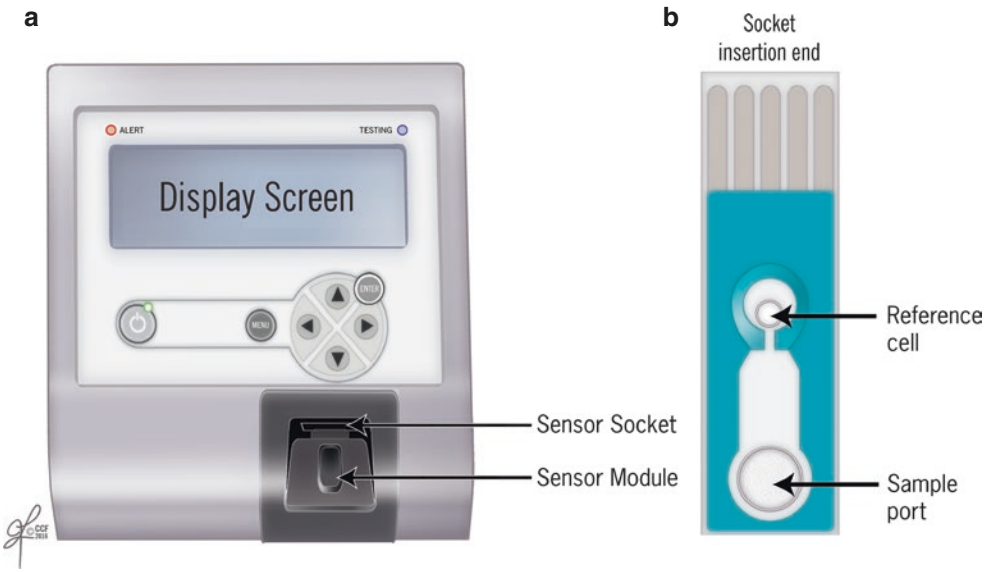


**Fig. 30.3** (**a**) The MiOXSYS instrument is a compact and simple instrument. (**b**) Due to the use of disposable sensors, semen samples can be applied to the sample port and through capillary action connect the reference cell to a working circuit. Once these circuits are connected, the instrument assesses the resistance of the sample and generates an ORP value. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013–2018. All Rights Reserved)

$$E(\mathrm{ORP}) = \frac{E^0 - \mathrm{R}\cdot\mathrm{T}}{n\cdot\mathrm{F}\cdot\ln\dfrac{[\mathrm{Ox}]}{[\mathrm{Red}]}} \qquad (30.1)$$

$E$ (ORP) is the oxidation-reduction potential of the sample (in mV), $E^0$ is the standard reduction potential of hydrogen, $n$ is the number of electrons exchanged (in moles), T is the absolute temperature (in degrees kelvin), F is Faraday's constant, R is the universal gas constant, [Ox] is the concentration of oxidations in the sample (in moles), and [Red] is the concentration of reductants in the sample (in moles).

The MiOXSYS System utilizes two primary components: a disposable single use platinum based sensor and a small galvanostat analyzer. The test sensors allow 30 μL of sample which is applied to the sensor port and connects the working circuit to the reference circuit [83]. The MiOXSYS analyzer is compact and requires very little laboratory bench space. The MiOXSYS system procedure takes approximately 5 minutes and requires very limited hands on time. The MiOXSYS System applies a low voltage current to the sample and the electron activity is measured in millivolts (mV). Results from the MiOXSYS system require the ORP to be normalized by the concentration [77].

## 30.4 Oxidation-Reduction Potential and Male Infertility

While other methods determine OS either by measuring ROS, TAC, or both, ORP is an independent measure of OS. Oxidation-reduction potential cutoff values were validated as a clinical marker of OS in infertile males (reviewed in Table 30.2). The first study was to establish if ORP can determine semen quality according to the WHO fifth edition guidelines.

### 30.4.1 Semen Quality and Oxidation-Reduction Potential

Prior to the diagnostic validation of the MiOXSYS system, a series of analytical studies were undertaken to determine the reliability and repeatability in a clinical laboratory setting. Agarwal et al. [77] established the ORP does not significantly vary between the time of collection and 120 min after liquefaction, validating ORP is stable in semen up to 2 h. In addition to the stability of time and ORP, the difference in ORP between semen and seminal plasma was determined. Agarwal et al. [43] validated that both semen and seminal plasma were time-independent and had ORP values correlating with each other.

Additional investigations examining the influence of oxidants on biological semen samples being analyzed by the MiOXSYS System were performed. Agarwal et al. studied the effects of cumene hydroperoxide in fresh and frozen semen samples. The study also investigated whether freezing of semen samples induced exogenous OS, which increased ORP [87]. Both motility and viability decreased significantly in a dose-dependent manner when exposed to cumene hydroperoxide. The ORP readings increased significantly in a dose-dependent manner, as well, but failed to show a significant correlation with the decrease in motility and viability [87]. In addition, it was validated that an increase in ORP is indicative of OS.

**Table 30.2** Determining a cutoff value for ORP to diagnosis male infertility

| ORP cutoff ($mV/10^6$ sperm/mL) | Pathologic indication | Sensitivity | Specificity | PPV | References |
|---|---|---|---|---|---|
| 1.635 | > 4 abnormalities on semen analysis | | | | [91] |
| 3.29 | Abnormal morphology of < 4% | | 89.1 | 85.7 | [92] |
| 1.57 | Able to detect at least one abnormality on semen analysis | | | | [109] |
| 1.42 | one abnormality on semen analysis across nine different centers | 97.1 | 43.7 | 94.2 | [116] |
| 1.48 | Abnormality on semen samples from a neat semen sample | 60 | 75 | | [77] |
| 2.09 | Abnormality on semen analysis with seminal plasma | 46.7 | 81.8 | | [77] |
| 1.57 | Detect one abnormality and able to determine oligozoospermic with greatest accuracy among patients | 70.4 | 88.1 | 95.5 | [105] |
| 1.38 | One abnormality on semen analysis with a better odds ratio than 1.41 | 63.3 | 87.8 | 97.6 | [89] |

*ORP* oxidation-reduction potential, *PPV* positive predictive value

Initial clinical validation of ORP was performed using semen samples from fertile donors and infertile patients. Cutoff value of 1.36 $mV/10^6$ sperm/ml was established with a sensitivity 69.6%, specificity 83.1%, positive predictive value 85.3% and negative predictive value 65.9% using MiOXSYS analyzer [106]. ORP levels were lower in fertile controls (1.03 $mV/10^6$ sperm/mL) compared with infertile patients (5.49 $mV/10^6$ sperm/mL) [89]. In fertile donors, ORP values were negatively correlated with sperm concentration [77]. Whereas, in infertile patients, ORP values were negatively correlated with both concentration ($r = -0.883$) and total motility ($r = -0.369$) [43]. Arafa et al. also reported a negative correlation between the ORP levels and semen parameters such as sperm concentration, total sperm count, total motility, progressive motility, and normal sperm morphology [89, 90]. Poor semen parameters with increased ORP indicates a state of OS.

Elbardisi et al. [91] divided patients into those who had an abnormality in at least one semen parameter ($n = 364$) and those with no abnormalities on semen analysis ($n = 64$) according to WHO fifth edition guidelines. ORP readings from the seminal plasma between abnormal and normal semen parameters revealed ORP did not correlate with motility but did so with progressive motility and morphology [91–94]. Seminal plasma ORP was most effective at differentiating patients with >4 abnormalities and was not effective in patients with a single abnormality on semen analysis [91]. Majzoub et al. [93] determined ORP values between infertile patients who had a significant correlation between abnormal morphology, particularly sperm head defects ($r = 0.34$), and SDF (measured by sperm chromatin dispersion assay; $r = 0.73$). Arafa et al. [95] assessed ORP's ability to correlate semen parameters and SDF. SDF was evaluated by Halosperm and significantly correlated with ORP ($r = 0.351$). Interestingly, patients with lower ORP values had higher total motility [95]. Subsequently, ORP correlated significantly with SDF values, it serves as a surrogate marker for centers without the capabilities to determine SDF [96].

While ORP comparisons between fertile and infertile patients assess how semen quality varies between patients of all etiologies to fertile controls, other studies determined if the MiOXSYS system can differentiate ORP values from patients with known OS-induced pathologies. Semen cryopreservation diminishes post-thaw sperm survival due to cryo-injury which is partially caused by OS induced by the increased metabolism during the thawing process [97]. The post-thawed semen samples produced higher ORP values which negatively correlated with post-thaw total motility, total semen counts, and cryo-survival [98]. Roychoudhury et al. [99] determined that grade 3 varicocele had significantly higher ORP levels compared to other varicocele grades in idiopathic infertile patients and fertile controls. Saleh and Agarwal [100] confirmed this difference between varicocele and idiopathic infertility. In addition, patients with varicocele had ORP levels which correlated with total motility, total motile count, progressive motility, abnormal morphology, and leukocyte concentration [100]. Varicocelectomy improves OS markers in male infertility patients [101–103], and, as expected, ORP reflects this in post-surgery patients [104]. Patients who had undergone varicocelectomy had significantly reduced ORP three months post-operation [104]. For patients with leukocytospermia, empiric doxycycline treatment reduced ORP by 56% after completion of the treatment [105]. With ORP reflecting poor semen quality, investigations were pursued on establishing various cutoff values to diagnose different aspects of male infertility.

### 30.4.2 Determining a Cutoff to Diagnose Infertile Men

In determining a cutoff value for the MiOXSYS system, a fundamental question prior to establishing a value is how many readings are required to produce a reliable result. To produce a reliable result, the MiOXSYS system requires a single reading. ORP values determined in duplicates change insignificantly by 0.1 $mV/10^6$ sperm/mL, indicating one reading is accurate [89]. Assaying how the observer can induce a change in the reading is an important aspect of any clinical test as readings can vary between a single

observer (intraobserver) and multiple observers (interobserver). Readings between a single observer and multiple observers vary by 3.61% by 8.39%, respectively, validating that the MiOXSYS system produces a reproducible and reliable diagnostic marker [106, 107]. Clinicians are reported with only a single ORP reading.

A cutoff ORP value for men with infertility versus fertile controls can be determined in a multitude of ways (reviewed in Table 30.2). One method to determine an ORP cutoff is to compare its values from fertile men and infertile men and establish a cutoff which distinguishes with the greatest accuracy between those two populations. Agarwal et al. [77] determined such a cutoff by evaluating both semen samples and seminal plasma from fertile and infertile men. The cutoff value for semen is 1.48 mV/$10^6$ sperm/mL with an accuracy of 78.9%. However, there is a difference between semen and seminal plasma cutoffs as seminal plasma cutoff is 2.09 mV/$10^6$ sperm/mL with an accuracy of 72.9%.

Reports have examined a second method to determine an ORP cutoff which can distinguish between semen without an abnormality and those with an abnormality according to WHO fifth edition guidelines [108]. When comparing men with completely normal semen parameters to men with at least one abnormality on semen analysis, an initial cutoff of >1.57 mV/$10^6$ sperm/mL indicates a patient has one abnormality on semen analysis and has OS [105, 109]. The cutoff values have a sensitivity of 70.4%, specificity of 88.1%, and a positive predictive value (PPV) of 95.5% to distinguish between semen with no abnormality and semen with at least one abnormality. Further refinement by Arafa et al. [110] determined a cutoff of 1.42 mV/$10^6$ sperm/mL that distinguishes infertile men with at least one abnormality with a specificity of 78% and PPV of 95.7%. A cutoff of 1.64 mV/$10^6$ sperm/mL differentiates 96% of infertility patients with greater than four abnormalities on semen analysis [91].

For a more specific cutoff value, ORP values which can distinguish abnormal morphology were further investigated as morphology has the highest predictive power to differentiate between infertile and fertile men [108]. A cutoff of 3.29 mV/$10^6$ sperm/mL distinguishes semen with high abnormal morphology [92]. When the sample size was increased from 400 infertile men to 1168, the cutoff was refined to 1.73 mV/$10^6$ sperm/mL with a sensitivity of 76%, specificity of 72%, and PPV of 69.2% [111].

To determine which method to utilize for a definitive cutoff value, Arafa et al. [89] compared fertile and infertile men. A cutoff of 1.36 mV/$10^6$ sperm/mL was determined with ORP from men with one abnormality on semen analysis. A second cutoff was determined by comparing infertile versus fertile ORP values regardless of semen analysis results which produced a cutoff of 1.41 mV/$10^6$ sperm/mL. By comparing which cutoff had a better odds ratio in distinguishing fertile from infertile men, the more accurate cutoff was determined as the ORP with an abnormality on semen analysis [89]. An ORP based on a semen abnormality by semen analysis can differentiate between normozoospermic and oligozoospermic men but cannot differentiate with asthenozoospermic men [108]. The cutoff of 1.36 mV/$10^6$ sperm/mL was further evaluated and has a sensitivity of 69.6%, specificity of 83.1%, and PPV of 85.3% [106]. Men with ORP values greater than 1.36 mV/$10^6$ sperm/mL had negatively correlated concentration ($r = -0.823$), total count ($r = -0.728$), motility ($r = -0.485$), and normal morphology ($r = -0.238$). While the cutoff value was determined, validation with more than one individual infertility center is required to validate the utility on a global scale.

### 30.4.3 Global Validation

The first multi-center was completed by a center in the USA and in Qatar. The main objective was to determine the ORP values in infertile men and fertile donors at both the centers and to establish a cutoff value. Both populations had a significant difference between fertile controls and infertile patients in concentration, motility, progressive motility, and normal morphology [112, 113]. To determine a cutoff, patients and controls were divided with the presence of one abnormality on semen analysis. Interestingly, the study found a difference between normal morphology and motility between infertile patients, validating the criticism of subjectivity in semen analysis [114, 115]. The cutoff was 2.26 mV/$10^6$ sperm/mL for the United States and 1.42 mV/$10^6$ sperm/mL for Qatar. When the two populations were combined, the cutoff was determined to be 1.42 mV/$10^6$ sperm/mL [112]. Infertility centers from the United States, Qatar, Japan, the United Kingdom, Turkey, Egypt, and India (nine in total) determined a cutoff of 1.34 mV/$10^6$ sperm/mL [112]. The data from the centers revealed ORP has the highest predictive value to differentiate fertile from infertile men, compared to traditional semen analysis [116].

### 30.4.4 Oxidation-Reduction Potential and In Vitro Fertilization

Continuing this trend, two studies evaluated whether ORP can determine the effect of various culture media on sperm. To determine if culture medium responses to OS are distinguishable by ORP, Panner Selvam et al. added varying concentrations of ascorbic acid and cumene hydroperoxide to culture media [117]. The effect on media shows ascorbic acid reduced ORP and cumene hydroperoxide increased

ORP. When comparing baseline ORP values of various media, a trend emerged; ART media had lower ORP values than sperm wash media and cryopreservation media had ORP values in between [117]. The findings suggest the antioxidant capacity of ART media is higher than that of cryopreservation and sperm wash media. Comparing the effect of ART media versus a neat semen sample after 1 h revealed polyvinylpyrrolidone (PVP) had a lower ORP than either hyaluronic acid or neat semen [118]. The results show PVP allows for better antioxidant capacity over a prolonged time compared to neat semen.

## 30.5 Five-Year Outlook

While the MiOXSYS system provides a novel method to determine OS, the system requires further validation within the IVF setting prior to universal acceptance.

Subsequently, the effect of ART media and semen is interesting; however, determining the physiological ORP of sperm will allow a more comprehensive understanding of how changes in ORP affect sperm. Understanding how media causes semen to deviate from physiological ORP allows the development of media which maintain semen quality longer. Subsequently, media refinement would minimize the effect of OS on successful fertilization after cryopreservation and assisted reproductive technique.

Oxidation-reduction potential correlates with SDF determined using Halosperm assay. As different methods to evaluate DNA fragmentation assess different aspects of SDF, investigating if ORP correlates with TUNEL and/or SCSA is required [119, 120]. This will better reassure the robustness of ORP as a surrogate marker for SDF.

## 30.6 Conclusion

OS and idiopathic male infertility has been well documented in the literature. Evaluating redox balance in a patient's semen has been explored previously, but the techniques are labor intensive, time-consuming, and unable to be standardized in routine clinical practice. The MiOXSYS System provides a rapid and accurate measure of redox balance in a simple and standardizable method. The MiOXSYS system cutoff based upon semen analysis abnormality produces the highest predictive power to distinguish between fertile and infertile men.

## 30.7 Review Criteria

An extensive review of the literature regarding OS, male infertility, and oxidation-reduction potential was performed using PubMed, Google Scholar, and Medline databases. Articles were initially assessed for the following keywords: "oxidation-reduction potential," "MiOXSYS," "male infertility," "semen analysis," "sperm functionality," and "oxidative stress." Only articles published in English were reviewed.

## References


1. Khatun A, Rahman MS, Pang M-G. Clinical assessment of the male fertility. Obstet Gynecol Sci. 2018;61(2):179.
2. Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertil Steril. 2014 Dec;102(6):1502–7.
3. Lewis SEM. Is sperm evaluation useful in predicting human fertility? Reproduction. 2007 Jul 1;134(1):31–40.
4. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001;345(19):1388–93.
5. Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol. 2016 Dec;5(6):935–50.
6. Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int Braz J Urol. 2014 Aug;40(4):433–53.
7. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol [Internet]. 2015 Dec [cited 2018 Jun 11];13(1). Available from: http://www.rbej.com/content/13/1/37.
8. Bungum M, Bungum L, Giwercman A. Sperm chromatin structure assay (SCSA): a tool in diagnosis and treatment of infertility. Asian J Androl. 2011 Jan;13(1):69–75.
9. Cummins JM, Pember SM, Jequier AM, Yovich JL, Hartmann PE. A test of the human sperm acrosome reaction following ionophore challenge: relationship to fertility and other seminal parameters. J Androl. 1991;12(2):98–103.
10. Pampiglione JS, Tan S-L, Campbell S. The use of the stimulated acrosome reaction test as a test of fertilizing ability in human spermatozoa. Fertil Steril. 1993 Jun;59(6):1280–4.
11. Oehninger S, Blackmore P, Morshedi M, Sueldo C, Acosta AA, Alexander NJ. Defective calcium influx and acrosome reaction (spontaneous and progesterone-induced) in spermatozoa of infertile men with severe teratozoospermia (Presented in part at the 18th annual meeting of the American Society of Andrology, Tampa, Florida, April 16 to 19, 1993). Fertil Steril. 1994 Feb;61(2):349–54.
12. Calvo L, Vantman D, Banks SM, Tezon J, Koukoulis GN, Dennison L, et al. Follicular fluid-induced acrosome reaction distinguishes a subgroup of men with unexplained infertility not identified by semen analysis. Fertil Steril. 1989 Dec;52(6):1048–54.
13. Liu DY, Clarke GN, Lopata A, Johnston WIH, Baker HG. A sperm-zona pellucida binding test and in vitro fertilization. Fertil Steril. 1989;52(2):281–7.
14. Grunewald S, Said TM, Paasch U, Glander H-J, Agarwal A. Relationship between sperm apoptosis signalling and oocyte penetration capacity. Int J Androl. 2008 Jun;31(3):325–30.
15. Grunewald S, Baumann T, Paasch U, Glander H-J. Capacitation and acrosome reaction in nonapoptotic human spermatozoa. Ann N Y Acad Sci. 2006 Dec 1;1090(1):138–46.
16. Signorelli J, Diaz ES, Morales P. Kinases, phosphatases and proteases during sperm capacitation. Cell Tissue Res. 2012 Sep;349(3):765–82.
17. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79(4):829–43.

18. Aitken RJ, Harkiss D, Buckingham DW. Analysis of lipid peroxidation mechanisms in human spermatozoa. Mol Reprod Dev. 1993 Jul;35(3):302–15.
19. Pasqualotto FF, Sundaram A, Sharma RK, Borges E, Pasqualotto EB, Agarwal A. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. Fertil Steril. 2008 Mar;89(3):602–7.
20. Sharma R, Roychoudhury S, Alsaad R, Bamajbuor F. Negative effects of oxidative stress (OS) on reproductive system at cellular level. In: Agarwal A, Sharma R, Gupta S, Harlev A, Ahmad G, du Plessis SS, et al., editors. Oxidative stress in human reproduction [Internet]. Cham: Springer International Publishing; 2017 [cited 2018 Jun 11]. p. 65–87. Available from: http://link.springer.com/10.1007/978-3-319-48427-3_4.
21. MacLeod J. The role of oxygen in the metabolism and motility of human spermatozoa. Am J Physiol-Leg Content. 1943;138(3):512–8.
22. Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C. Reactive oxygen species and sperm physiology. Rev Reprod. 1997;2(1):48–54.
23. de Lamirande E, O'Flaherty C. Sperm activation: role of reactive oxygen species and kinases. Biochim Biophys Acta. 2008 Jan;1784(1):106–15.
24. Aitken R. Molecular mechanisms regulating human sperm function. Mol Hum Reprod. 1997 Mar 1;3(3):169–73.
25. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009 Jan 1;417(1):1–13.
26. Cho C-L, Esteves S, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl. 2016;18(2):186.
27. Agarwal A, Prabakaran S, Allamaneni S. What an andrologist/urologist should know about free radicals and why. Urology. 2006;67(1):2–8.
28. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: an updated review of literature. Arab J Urol. 2018 Mar;16(1):35–43.
29. Doshi SB, Khullar K, Sharma RK, Agarwal A. Role of reactive nitrogen species in male infertility. Reprod Biol Endocrinol. 2012;10(1):109.
30. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Indian J Exp Biol. 2005;43(1):963–74.
31. Agarwal A, Ahmad G, Sharma R. Reference values of reactive oxygen species in seminal ejaculates using chemiluminescence assay. J Assist Reprod Genet. 2015 Dec;32(12):1721–9.
32. Morrison CD, Brannigan RE. Metabolic syndrome and infertility in men. Best Pract Res Clin Obstet Gynaecol. 2015 May;29(4):507–15.
33. Bachir BG, Jarvi K. Infectious, inflammatory, and immunologic conditions resulting in male infertility. Urol Clin North Am. 2014 Feb;41(1):67–81.
34. Comhaire F. Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist's viewpoint. Hum Reprod Update. 1999 Sep 1;5(5):393–8.
35. Ochsendorf FR. Infection and reactive oxygen species. Andrologia. 2009 Apr 27;30(S1):81–6.
36. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ Jr. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril. 2002;78(3):491–9.
37. Adams JA, Galloway TS, Mondal D, Esteves SC, Mathews F. Effect of mobile telephones on sperm quality: a systematic review and meta-analysis. Environ Int. 2014 Sep;70:106–12.
38. De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. Zhang B, editor. PLoS One. 2009 Jul 31;4(7):e6446.
39. Evenson DP, Jost LK, Corzett M, Balhorn R. Characteristics of human sperm chromatin structure following an episode of influenza and high fever: a case study. J Androl. 2000;21(5):739–46.
40. Agarwal A, Said TM, Bedaiwy MA, Banerjee J, Alvarez JG. Oxidative stress in an assisted reproductive techniques setting. Fertil Steril. 2006 Sep;86(3):503–12.
41. Gagnon C, Iwasaki A, Lamirande E, Kovalski N. Reactive oxygen species and human spermatozoa. Ann N Y Acad Sci. 1991 Dec;637(1 The Male Germ):436–44.
42. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxidative Med Cell Longev. 2008;1(1):15–24.
43. Pasqualotto FF, Sharma RK, Pasqualotto EB, Agarwal A. Poor semen quality and ROS-TAC scores in patients with idiopathic infertility. Urol Int. 2008;81(3):263–70.
44. Egeberg Palme DL, Rehfeld A, Bang AK, Nikolova KA, Kjærulff S, Petersen MR, et al. Viable acrosome-intact human spermatozoa in the ejaculate as a marker of semen quality and fertility status. Hum Reprod. 2018 Mar 1;33(3):361–71.
45. Rengan AK, Agarwal A, van der Linde M, du Plessis SS. An investigation of excess residual cytoplasm in human spermatozoa and its distinction from the cytoplasmic droplet. Reprod Biol Endocrinol. 2012;10(1):92.
46. Baker MA, Krutskikh A, Curry BJ, Hetherington L, Aitken RJ. Identification of cytochrome-b5 reductase as the enzyme responsible for NADH-dependent lucigenin chemiluminescence in human Spermatozoa. Biol Reprod. 2005 Aug 1;73(2):334–42.
47. Henkel RR. Leukocytes and oxidative stress: dilemma for sperm function and male fertility. Asian J Androl. 2011 Jan;13(1):43–52.
48. Saleh RA, Agarwal A, Kandirali E, Sharma RK, Thomas AJ Jr, Nada EA, et al. Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. Fertil Steril. 2002;78(6):1215–24.
49. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32(1):1.
50. Aitken R. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev. 1995;7(4):659.
51. Jones R, Mann T. Lipid peroxidation in spermatozoa. Proc R Soc B Biol Sci. 1973 Aug 31;184(1074):103–7.
52. Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. Fertil Steril. 1979;31(5):531–7.
53. Aitken RJ, Gibb Z, Mitchell LA, Lambourne SR, Connaughton HS, De Iuliis GN. Sperm motility is lost in vitro as a consequence of mitochondrial free radical production and the generation of electrophilic aldehydes but can be significantly rescued by the presence of nucleophilic thiols. Biol Reprod. 2012;87(5):110.
54. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial Metabolism of Reactive Oxygen Species. Biochemistry (Mosc). 2005;70(2):15.
55. Wang X, Sharma RK, Gupta A, George V, Thomas AJ, Falcone T, et al. Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. Fertil Steril. 2003 Sep;80:844–50.
56. Ruiz-Pesini E, Diez C, Montoya J, Alvarez E, Arenas J. Correlation of sperm motility with mitochondrial enzymatic activities. Clin Chem. 1998;8:5.
57. Mahfouz R, Sharma R, Thiyagarajan A, Kale V, Gupta S, Sabanegh E, et al. Semen characteristics and sperm DNA fragmentation in infertile men with low and high levels of seminal reactive oxygen species. Fertil Steril. 2010 Nov;94(6):2141–6.
58. Lopes S, Jurisicova A, Sun J-G, Casper RF. Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa. Hum Reprod. 1998;13(4):896–900.
59. Aitken RJ, De Iuliis GN, Finnie JM, Hedges A, McLachlan RI. Analysis of the relationships between oxidative stress, DNA damage and sperm vitality in a patient population: development of diagnostic criteria. Hum Reprod. 2010 Oct 1;25(10):2415–26.
60. Xie D, Lu C, Zhu Y, Zhu S, Yang E-J, Jin X. Analysis on the association between sperm DNA fragmentation index and conventional

semen parameters, blood microelements and seminal plasma ROS in male patients with infertility. Exp Ther Med [Internet]. 2018 May 2 [cited 2018 Aug 6]. Available from: http://www.spandidos-publications.com/10.3892/etm.2018.6115.
61. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online. 2006 Jan;12(4):466–72.
62. Muriel L, Garrido N, Fernández JL, Remohí J, Pellicer A, de los Santos MJ, et al. Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril. 2006 Feb;85(2):371–83.
63. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010 Mar;93(4):1027–36.
64. Agarwal A, Allamaneni SS, Said TM. Chemiluminescence technique for measuring reactive oxygen species. Reprod Biomed Online. 2004 Jan;9(4):466–8.
65. Homa ST, Vessey W, Perez-Miranda A, Riyait T, Agarwal A. Reactive oxygen species (ROS) in human semen: determination of a reference range. J Assist Reprod Genet. 2015 May;32(5):757–64.
66. Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil Steril. 1995;64(4):868–70.
67. Said TM, Kattal N, Sharma RK, Sikka SC, Thomas AJ, Mascha E, et al. Enhanced chemiluminescence assay vs colorimetric assay for measurement of the total antioxidant capacity of human seminal plasma. J Androl. 2003 Sep 10;24(5):676–80.
68. Esfandiari N, Sharma RK, Saleh RA, Thomas AJ, Agarwal A. Utility of the nitroblue tetrazolium reduction test for assessment of reactive oxygen species production by seminal leukocytes and spermatozoa. J Androl. 2003 Nov 12;24(6):862–70.
69. Gosálvez J, Coppola L, Fernández JL, López-Fernández C, Góngora A, Faundez R, et al. Multi-centre assessment of nitroblue tetrazolium reactivity in human semen as a potential marker of oxidative stress. Reprod Biomed Online. 2017 May;34(5):513–21.
70. McKinney KA, Lewis SEM, Thompson W. Reactive oxygen species generation in human sperm: luminol and lucigenin chemiluminescence probes. Arch Androl. 1996 Jan;36(2):119–25.
71. Baumber J, Ball BA, Gravance CG, Medina V, Davies-Morel MCG. The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. J Androl. 2000;21(6):895–902.
72. Sharma RK, Sabanegh E, Mahfouz R, Gupta S, Thiyagarajan A, Agarwal A. TUNEL as a test for sperm DNA damage in the evaluation of male infertility. Urology. 2010 Dec;76(6):1380–6.
73. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 1993 Apr;84(4):407–12.
74. Agarwal A, Bui AD. Oxidation-reduction potential as a new marker for oxidative stress: correlation to male infertility. Investig Clin Urol. 2017;58(6):385.
75. Agarwal A, Majzoub A. Laboratory tests for oxidative stress. Indian J Urol. 2017;33(3):199.
76. Amarasekara DS, Wijerathna S, Fernando C, Udagama PV. Cost-effective diagnosis of male oxidative stress using the nitroblue tetrazolium test: useful application for the developing world. Andrologia. 2014 Mar;46(2):73–9.
77. Agarwal A, Sharma R, Roychoudhury S, Du Plessis S, Sabanegh E. MiOXSYS: a novel method of measuring oxidation reduction potential in semen and seminal plasma. Fertil Steril. 2016 Sep;106(3):566–573.e10.
78. Hickling A. Studies in electrode polarisation. Part IV—the automatic control of the potential of a working electrode. Trans Faraday Soc. 1942;38(1):27–34.
79. Champ DR, Gulens J, Jackson RE. Oxidation–reduction sequences in ground water flow systems. Can J Earth Sci. 1979 Jan;16(1):12–23.
80. Suslow TV. Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation [Internet]. University of California, Agriculture and Natural Resources; 2004 [cited 2018 Jun 14]. Available from: http://anrcatalog.ucanr.edu/Details.aspx?itemNo=8149.
81. Kim C, Hung Y-C, Brackett RE. Roles of oxidation–reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. J Food Prot. 2000 Jan;63(1):19–24.
82. Jellinek M. Oxidation-reduction maintenance in organ preservation. Arch Surg. 1985 Apr 1;120(4):439.
83. Rael LT, Bar-Or R, Kelly MT, Carrick MM, Bar-Or D. Assessment of oxidative stress in patients with an isolated traumatic brain injury using disposable electrochemical test strips. Electroanalysis. 2015 Nov;27(11):2567–73.
84. Spanidis Y, Goutzourelas N, Stagos D, Mpesios A, Priftis A, Bar-Or D, et al. Variations in oxidative stress markers in elite basketball players at the beginning and end of a season. Exp Ther Med. 2016 Jan;11(1):147–53.
85. Spanidis Y, Stagos D, Orfanou M, Goutzourelas N, Bar-Or D, Spandidos D, et al. Variations in oxidative stress levels in 3 days follow-up in ultramarathon mountain race athletes. J Strength Cond Res. 2017 Mar;31(3):582–94.
86. Spanidis Y, Mpesios A, Stagos D, Goutzourelas N, Bar-Or D, Karapetsa M, et al. Assessment of the redox status in patients with metabolic syndrome and type 2 diabetes reveals great variations. Exp Ther Med. 2016 Mar;11(3):895–903.
87. Agarwal A, Sharma R, Henkel R, Roychoudhury S, Sikka SC, du Plessis S, et al. Cumene hydroperoxide induced changes in oxidation-reduction potential in fresh and frozen seminal ejaculates. Andrologia. 2018 Feb;50(1):e12796.
88. Agarwal A, Sharma R, Du Plessis S, Sabanegh E. Validation of measuring oxidation-reduction potential in fresh and frozen semen samples with Mioxsys diagnostic system. J Urol. 2016 Apr 1;195(4):e914.
89. Arafa M, Agarwal A, Al Said S, Majzoub A, Sharma R, Bjugstad KB, et al. Semen quality and infertility status can be identified through measures of oxidation-reduction potential. Andrologia. 2018 Mar;50(2):e12881.
90. Arafa M, Majzoub A, Agarwal A, Henkel R, Eliwa J, AlSaid S, et al. Oxidation reduction potential is correlated to spermatogenic testicular function in infertile men. Hum Reprod. 2018;33(suppl_1):i167.
91. Elbardisi H, Arafa M, Agarwal A, AlSaid S, Majzoub A, Keyvanifard F, et al. Seminal fluid static oxidation reduction potential is lower in seminal fluid that meets WHO criteria for fertile men. Hum Reprod. 2016;31(suppl_1):i135.
92. Arafa M, Agarwal A, Elbardisi H, AlSaid S, Majzoub A, Keyvanifard F, et al. Oxidation reduction potential: a new predictor for sperm morphology in infertile men. Hum Reprod. 2016;31(suppl_1):i136.
93. Majzoub A, Arafa M, Elbardisi H, Al Said S, Agarwal A, Al Rumaihi K. Oxidation reduction potential and sperm DNA fragmentation levels in sperm morphologic anomalies. Hum Reprod. 2017;32(suppl_1):i61.
94. Elbardisi H, Arafa M, Agarwal A, AlSaid S, AlRumaihi K, AlAnsari A, et al. Oxidation reduction potential in male infertility management: a report of 4080 cases. Fertil Steril. 2018 Sep 1;110(4):e159.
95. Arafa M, Elbardisi H, Majzoub A, AlSaid S, AlNawasra H, Khalafalla K, et al. Correlation of sperm DNA fragmentation and seminal oxidation reduction potential in infertile men. BioMedicine. 2017;1(26):68–78.
96. Agarwal A, Arafa MM, Elbardisi H, Majzoub A, Alsaid SS. Relationship between seminal oxidation reduction potential

and sperm DNA fragmentation in infertile men. Fertil Steril. 2017 Sep 1;108(3):e316.
97. Thomson LK, Fleming SD, Aitken RJ, De Iuliis GN, Zieschang J-A, Clark AM. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum Reprod. 2009 Sep 1;24(9):2061–70.
98. Saleh R, Agarwal A, Elsuity M. High seminal oxidation reduction potential in cryopreserved semen from infertile men is a marker of poor post-thaw sperm quality. Fertil Steril. 2017 Sep 1;108(3):e317.
99. Agarwal A, Majzoub A, Roychoudhury S, Arafa MM. Oxidation reduction potential: a novel marker of varicocele pathophysiology. Fertil Steril. 2016 Sep 1;106(3):e294–5.
100. Saleh R, Agarwal A. High levels of seminal oxidation reduction potential (ORP) in infertile men with clinical varicocele. Hum Reprod. 2017;32(suppl_1):i169.
101. Cervellione RM, Cervato G, Zampieri N, Corroppolo M, Canoglio F, Cestaro B, et al. Effect of varicocelectomy on the plasma oxidative stress parameters. J Pediatr Surg. 2006 Feb;41(2):403–6.
102. Mancini A, Milardi D, Bianchi A, Festa R, Silvestrini A, Marinis LD, et al. Increased total antioxidant capacity in seminal plasma of varicocele patients: a multivariate analysis. Arch Androl. 2007 Jan;53(1):37–42.
103. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. Int J Androl. 2001 Oct 21;24(5):261–5.
104. Elbardisi H, Arafa M, Agarwal A, AlSaid A, AlRumaihi K, AlAnsari A, et al. Effect of varicocelectomy on ORP in varicocele associated male infertility. Hum Reprod. 2018;33(suppl_1):i166.
105. Agarwal A, Wang SM. Clinical relevance of oxidation-reduction potential in the evaluation of male infertility. Urology. 2017 Jun;104:84–9.
106. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. Reprod Biomed Online. 2017 Jan;34(1):48–57.
107. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Bjugstad K, et al. Evaluation of intra- and inter-observer reliability of the ORP (oxidation-reduction potential) test for OS (oxidative stress) in male factor infertility. Fertil Steril. 2016 Sep 1;106(3):e300–1.
108. Agarwal A, Henkel R, Sharma R, Tadros NN, Sabanegh E. Determination of seminal oxidation-reduction potential (ORP) as an easy and cost-effective clinical marker of male infertility. Andrologia. 2018 Apr;50(3):e12914.
109. Agarwal A, Wang S, Gupta S, Sharma R, Sabanegh ES. Oxidation reduction potential is a surrogate marker of poor sperm quality. Fertil Steril. 2017 Sep 1;108(3):e316.
110. Arafa MM, Elbardisi H, Agarwal A, Majzoub A, Sharma R, Alsaid SS, et al. Seminal oxidation reduction potential can differentiate fertile from infertile men. Fertil Steril. 2016 Sep 1;106(3):e234.
111. Majzoub A, Arafa M, Mahdi M, Agarwal A, Al Said S, Al-Emadi I, et al. Oxidation–reduction potential and sperm DNA fragmentation, and their associations with sperm morphological anomalies amongst fertile and infertile men. Arab J Urol. 2018 Mar;16(1):87–95.
112. Agarwal A, Arafa M, Chandrakumar R, Majzoub A, AlSaid S, Elbardisi H. A multicenter study to evaluate oxidative stress by oxidation-reduction potential, a reliable and reproducible method. Andrology. 2017 Sep;5(5):939–45.
113. Agarwal A, Chandrakumar R, Arafa MM, Elbardisi H, Okada H, Suzuki K, et al. Multi-center evaluation of oxidation reduction potential assay in the infertile male. Fertil Steril. 2017 Sep 1;108(3):e317.
114. Cooper TG, Neuwinger J, Bahrs S, Nieschlag E. Internal quality control of semen analysis. Fertil Steril. 1992;58(1):172–8.
115. Neuwinger J, Behre HM, Nieschlag E. External quality control in the andrology laboratory: an experimental multicenter trial. Fertil Steril. 1990;54(2):308–14.
116. Agarwal A, Chandrakumar R, Arafa M, Elbardisi H, Okada H, Suzuki K, et al. Evaluating the predictability of oxidation-reduction potential in male factor infertility in conjunction with semen analysis: a multicenter study. Hum Reprod. 2018;33(suppl_1):i152.
117. Panner Selvam MK, Henkel R, Sharma R, Agarwal A. Calibration of redox potential in sperm wash media and evaluation of oxidation-reduction potential values in various assisted reproductive technology culture media using MiOXSYS system. Andrology. 2018 Mar;6(2):293–300.
118. Agarwal S, Roychoudhury S, Esteves I, Maldonado Rosas R, Sharma S. Gupta. Oxidation-reduction potential (ORP) of spermatozoa selected for intracytoplasmic sperm injection (ICSI) after exposure to polyvinylpyrrolidone (PVP) and hyaluronic acid (HA). 32nd Annual European Society for Health and Reproduction Conference, 2016;1: pp. 3–6.
119. Henkel R, Hoogendijk CF, Bouic PJD, Kruger TF. TUNEL assay and SCSA determine different aspects of sperm DNA damage: different tests for the determination of sperm DNA damage. Andrologia. 2010 Sep 22;42(5):305–13.
120. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, Abad C, Amengual MJ, Prada E, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral comet assay. Andrology. 2013 Sep;1(5):715–22.
121. Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. Fertil Steril. 2009 Mar;91(3):805–11.
122. Smith R, Vantman D, Ponce J, Escobar J, Lissi E. Andrology: total antioxidant capacity of human seminal plasma. Hum Reprod. 1996;11(8):1655–60.
123. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ, Agarwal A. The reactive oxygen species—total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod. 1999 Nov;14(11):2801–7.
124. Tunc O, Thompson J, Tremellen K. Development of the NBT assay as a marker of sperm oxidative stress: NBT assay of sperm oxidative stress. Int J Androl. 2010 Feb;33(1):13–21.
125. Halliwell B, Gutteridge JMC. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett. 1981 Jun 15;128(2):347–52.
126. Aitken RJ, Smith TB, Lord T, Kuczera L, Koppers AJ, Naumovski N, et al. On methods for the detection of reactive oxygen species generation by human spermatozoa: analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. Andrology. 2013 Mar;1(2):192–205.