Oxidation reduction potential: a new biomarker of male infertility

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ABSTRACT

Oxidative stress is considered a major etiology for male infertility, more specifically idiopathic infertility. The causes of seminal oxidative stress can be intrinsic, such as varicocele or due to the presence of active leukocytes and immature germ cells. Reported external causes are smoking, alcohol or exposure to environmental toxins. Traditional methods to determine the seminal oxidative stress do not evaluate this status directly, but rather measure its components or intermediate products indirectly, instead. The major disadvantages of the traditional methods are related with time and cost as these methods are extremely time consuming and require expensive equipment, consumables and highly skilled laboratory personnel. To overcome these drawbacks, the MiOXSYS® system, a method which directly measures the oxidation-reduction potential (ORP), was developed. The evaluation of the ORP using MiOXSYS® is cost-effective, easy and quick. However, this newly introduced method to evaluate the oxidative status of semen still requires validation in different andrology laboratory settings across the world.


KEY WORDS: Male infertility - Oxidation-reduction - Oxidative stress - Semen analysis.

Infertility is defined as failure to achieve a clinical pregnancy after one year of regular and unprotected intercourse.1 Globally, the prevalence of infertility in couples is about 15%, and is estimated between 2.5-12% of men are infertile, based on the geographic location.1 Among the female and male factors contributing to infertility it is estimated that the male contribution ranges from 20% up to 70% to the infertility of the couple.1 Male infertility is related to several factors, among them emotional, sociocultural and financial problems.2 In addition, many medical conditions such as varicocele, hypogonadotropic hypogonadism, disorders in ciliary function, cystic fibrosis, infection, systemic diseases, testicular deficiency, and post testicular impairment are also associated with male infertility. Further, unhealthy lifestyle choices and associated metabolic diseases are contributing to an increase in the incidence of male fertility.3

The basic semen analysis according to World Health Organization (WHO) guidelines is still the cornerstone of laboratory male fertility evaluation.4 The quality and thus the functionality of spermatozoa reflects the status of germ cells epithelium, epididymis and accessory sexual glands.5, 6 This basic semen analysis is far from perfect. Indeed, the reference values for semen analysis as established by the WHO 20104 do not differentiate the fertile from infertile men. In fact, this evaluation neither takes dysfunctions such as DNA fragmentation or the oxidative state of the ejaculate, nor genetic variability of spermatozoa, into account.7, 8 Moreover, the reference limits suggested by the WHO 2010 guidelines do not represent
In this review, we present an overview on the OS concepts as well as its causes and the mechanisms behind the overproduction of ROS. We discuss a new biomarker for the evaluation of OS by the measurement of oxidation-reduction potential (ORP), describe the test principle of this novel parameter and highlight the advantages and clinical significance of the assay.

Oxidative stress and spermatozoa

Reactive oxygen species

The production of ROS is essential for the homeostasis in aerobically living cells. However, sometimes the system is unable to neutralize an excessive production of ROS or exposure of cells to excessive amounts of ROS causing an imbalance between oxidants and antioxidants resulting in a state of OS. Typically, ROS are free radical oxygen derivatives. Radicals are molecules containing unpaired electrons in the outer orbit, a chemical condition, which renders these molecules electronically unstable and therefore highly reactive in order to reach stability. Other strongly oxidizing molecules such as H₂O₂, though not a free radical, or nitric oxide and the peroxynitrite anion have a role in oxidation-reduction reactions in fertility. The main targets for ROS and oxidants are electron-rich molecules which can easily be oxidized such as polyunsaturated lipids.

Figure 1.—The production of reactive oxygen species by the spermatozoa is essential for some physiological processes such as capacitation, acrosome reaction, hyperactivation and sperm-oocyte binding. However, when the ROS production overcome the antioxidant defenses of the spermatozoa due to endogenous or exogenous sources it can cause lipid peroxidation, protein oxidation, DNA damage or apoptosis in the male reproductive tract and lead to an infertility state.

In light of these problems, new test systems to evaluate sperm functional disorders and anomalies are needed to improve male fertility diagnostics. In fact, the American Society for Reproductive Medicine acknowledges the limitations of basic semen analysis and has included sperm function tests, such as single-cell gel electrophoresis assay (Comet), terminal deoxynucleotide transferase–mediated dUTP nick-end labeling (TUNEL) assay or the sperm chromatin structure assay (SCSA) into the evaluation of infertile men. However, measurement of OS and ROS by direct or indirect method are not included as these techniques are not properly evaluated yet, are not sensitive enough, or are too susceptible to interference.
Leukocytes are mainly responsible for the high production of ROS, which in turn are detrimental to male fertility. Yet, the effects of leukocytospermia on male fertility are controversially discussed.

Leukocytospermia has been related with impaired capacitation and sperm fertilizing capacity. However, although many studies reported a negative effect of leukocytospermia on semen quality or sperm DNA fragmentation, the connection between leukocytospermia and these processes is not well established. In addition, the incidence of leukocytospermia correlates only poorly with other semen parameters. The presence of leukocytes in semen can be a consequence of an infection, inflammation or cellular defense mechanisms in the male genital tract where leukocytes will be activated.

The mechanism of ROS generation in leukocytes is the same as in spermatozoa. However, in order to destroy the pathogens, leukocytes release large quantities of superoxide, which are about 1000 times more than that produced in spermatozoa. Due to their immunological defense function, leukocyte contribution to the overall ROS in semen is extremely high. If this overwhelms the limited antioxidant capacity of spermatozoa and seminal plasma, a stage of oxidative stress will occur.

Since spermatozoa are extremely prone to oxidative assaults because of their extraordinary high amount of polyunsaturated fatty acids (PUFAs) in their plasma membranes, membrane lipids will be oxidized in a process named lipid peroxidation. PUFAs contains more than two carbon-carbon double bonds, which are the primary site of the assault. Most PUFAs have unconjugated double bonds separated by methylene groups. Chemically, double bonds adjacent to a methylene group cause that the methylene carbon-hydrogen is more susceptible to abstraction. When the abstraction occurs, the radical that is formed is stabilized by rearrangement of the double bonds, which can be then oxidized by oxygen leading to peroxyl radical, which in turn can oxidize neighboring PUFAs in a radical chain reaction. The propagation of this process depends of the antioxidant capacity of the spermatozoa.

Leukocytospermia

Leukocytes are part of the ejaculated cells in semen and present in the male reproductive tract, even in healthy men. The main sources of peroxidase-positive leukocytes are the prostate and seminal vesicles. According to the WHO, more than 1×10^6 per milliliter of peroxidase-positive leukocytes are regarded as leukocytospermia.

Varicocele

Varicocele is a tortuosity and dilation of the veins of the Pampiniform plexus in the spermatic cord and a major cause for male infertility. Varicocele is described as a pathological cause for elevated OS and for a decrease in sperm quality. Indeed, men with varicocele have elevated OS, even when they are fertile. OS in seminal plasma is reported to increase with higher grades of varicocele. The mechanisms related to the increase of ROS or the decrease in antioxidant defense in case of varicoceles, however, are not well understood. A current hypothesis based on the generation of ROS due to testicular hypoxia, increase in scrotal temperature, epididymal dysfunction and accumulation of toxins. Testicular hypoxia was investigated as a cause of OS in men with varicocele by evaluating hypoxia-inducible factor-1α. This factor was over-expressed in the internal spermatic vein and/or is related to oxidative stress. An increase in testicular temperature impairs spermatogenesis and consequently decreases sperm quality. In fact, some studies showed an elevation in scrotal temperature of men with varicocele. Yet, theories on how varicocele increases temperature remain unclear.

Besides an improvement in sperm quality and pregnancy rates, a decrease in seminal oxidative stress in men after a varicocelectomy is well reported, more specifically a decrease in sperm DNA damage.

Immature spermatozoa

Besides leukocytes, immature spermatozoa are another source of ROS in the ejaculate. Here, the increase in ROS generation is linked to an increase in cytoplasmic droplets typically found in immature sperm. The increase in biomarkers of cytoplasmic space and lipid peroxidation was correlated with abnormalities in spermatozoa.
Environmental factors

Nowadays, many people are exposed to numerous environmental toxins as well as to cigarette smoke or excessive alcohol. The human body metabolizes alcohol and one of the products is NADH, a compound, which is responsible for the respiratory chain activity and for the increase in ROS formation. Consumption of alcohol is also associated with a state of hypoxia leading to lesions in the tissues. NADH and acetaldehyde are products of alcohol metabolism. Acetaldehyde is an intermediates in this process, which, when in contact with proteins and lipids also increases ROS production. Moreover, due to its reactive nature by reacting with proteins and lipids, acetaldehyde is damaging the mitochondria, which consequently results in decreased ATP production. NADH accumulation stimulates the activity of the respiratory chain, consuming the existing oxygen and is eventually forming ROS.

Many studies have reported low semen quality such as increase in morphologically abnormal sperm in alcoholic men. Smoking is another risk factor leading to excessive ROS production. Tobacco contains approximately 4000 harmful substances and cigarette smoke contains more than 7000 chemical compounds. Among these, many are known for their ability to increase ROS production, which is even more harmful for fertility as the presence of other byproducts in cigarette smoke such as cotinine and hydroxycotinine that are reported in seminal plasma. The effects of smoking on sperm quality are well documented with reports consistently showing decreased sperm quality i.e. sperm count, motility and viability and increased DNA damage.

Men are also unintentionally exposed to many other environmental toxins, such as radiation, pharmacological compounds or pollutants that can accumulate in the body and in the testes thereby increasing the ROS production. Studies have reported impairment of male fertility potential in a variety of cases. For example, pesticides and chemical fertilizers affect sperm count in farmers and increase sperm DNA damage. On the other hand, phthalates, present in most plastics as plasticizer, reportedly increase DNA sperm damage and reduce sperm motility.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Assay</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faulkner, Agarwal</td>
<td>Chemiluminescence</td>
<td>• High sensitivity and specificity</td>
<td>• Requires large and expensive equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Evaluates intra- and extracellular ROS</td>
<td>• Highly time-consuming</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Highly reproducible</td>
<td>• Requires a large amount of sample</td>
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<tr>
<td>Esfandiari</td>
<td>Nitroblue Tetrazolium</td>
<td>• Easy to perform</td>
<td>• Dependent of the half-lives of the probes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cost-effective method</td>
<td>• Low specificity (can occur cross reactions with oxidoreductases)</td>
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<td></td>
<td></td>
<td>• Can provide the source of ROS (light microscope)</td>
<td>• Subjective interpretation</td>
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<tr>
<td>Dikalov</td>
<td>Cytochrome C Reduction Test</td>
<td>Detects high levels of ROS</td>
<td>Does not detect O₂⁻ intracellular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Evaluates O₂⁻ released to the extracellular space</td>
<td>Low sensitivity to detect NADPH oxidase low activity</td>
</tr>
<tr>
<td>Kohno</td>
<td>Electron Spin Resonance</td>
<td>Detects high levels of ROS</td>
<td>Reducing agents presented in spin adduct can neutralize free radicals</td>
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<tr>
<td></td>
<td></td>
<td>• Characteristics of free radicals, formation and elimination velocities of free radicals</td>
<td>If the radical reacts immediately with other molecules will not be detected</td>
</tr>
<tr>
<td>Draper</td>
<td>Thiobarbituric Acid Assay</td>
<td>Inexpensive and simple method</td>
<td>Requires laborious standards</td>
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<tr>
<td></td>
<td></td>
<td>• Can be evaluated by fluorometry or spectrophotometry</td>
<td>Not used in clinical environment</td>
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<tr>
<td></td>
<td></td>
<td>• For low sperm concentrations sensitive HLPc can be used</td>
<td></td>
</tr>
<tr>
<td>Said, Whitehead</td>
<td>Total Antioxidant Capacity</td>
<td>High sensitivity and specificity</td>
<td>Requires large and expensive equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Measures the total antioxidant capacity of the sample</td>
<td>Highly time-consuming</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Highly reproducible</td>
<td>Requires a large amount of sample</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Limited to the half-lives of the probes</td>
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<td></td>
<td></td>
<td>Requires a trained operator</td>
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<td>Affected by the viscosity of the sample</td>
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<td></td>
<td>Requires further validation for outcome</td>
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<tr>
<td>Agarwal</td>
<td>MiOXSYS® system</td>
<td>Snapshot of the oxidative state of the sample</td>
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<tr>
<td></td>
<td></td>
<td>• Easy and simple to execute</td>
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<td></td>
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<td>• Inexpensive</td>
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<td>• Measures fresh and frozen samples</td>
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Cadmium is a heavy metal with similarities in its chemistry to the trace element zinc can be incorporated in the body where it accumulates with harmful effects of male fertility. Radiation is also not only related to an increase in seminal ROS production, but also in low sperm motility and DNA integrity. This includes electromagnetic radiations emitted by cell phones. Radiotherapy and chemotherapy used in cancer treatment causes azoospermia.

**OS measurement**

Since seminal OS has significant adverse effects on ejaculated spermatozoa, it is important to quantify the levels of OS in a given sample to obtain a more accurate picture of the seminal redox level and develop an optimized treatment plan for affected infertile men. The measurement of OS in the ejaculate can either be done directly by measuring OS, ROS or reactive nitrogen species, or indirectly by determining end products of lipid peroxidation (e.g. malondialdehyde [MDA]) or end products of ROS production, and cofactors and antioxidants. Advantages and disadvantages of the direct and indirect methods to evaluate OS are presented in Table I.

**Direct measurement of OS**

Methods for the direct measurement of ROS in semen include: 1) chemiluminescence; 2) nitro blue tetrazolium (NBT) test; 3) cytochrome c reduction test; and 4) electron spin resonance.

Chemiluminescent methods are most commonly used for the measurement of ROS in semen and spermatozoa. They evaluate both intra- and extracellular ROS, but not the damage caused by the ROS. The principle of these tests is based on the combination of a chemiluminescent probe with a free radical resulting in the emission of a light signal that is quantified in a luminometer. This test can use two different probes: luminol or lucigenin. Luminol is extremely sensitive at pH of 7 and reacts with the majority of ROS and the reactive radical form is generated by univalent oxidation. On the other hand, lucigenin only measures the superoxide radical present in the extracellular space. The radical formed is generated by univalent reduction.

The NBT test is used to evaluate neutrophil leukocyte function and quantify cellular oxidative metabolism. In this test, cells are incubated with NBT, which is reduced to water insoluble formazan crystals by a cytoplasmic oxidase system (superoxide ions) which helps to transfer electrons from NADPH to NBT. These formazan crystals can microscopically be evaluated. Alternatively, these crystals can be solubilized and the absorbance of the resulting purple-blue solution can be measured. The results of the NBT test reflects the ROS-generating activity in the cytoplasm and can detect the cellular source of ROS in samples such as semen.

The cytochrome c reduction test detects large quantities of the free radical superoxide (O$_2^-$) that is released into the extracellular space by the cells. The principle is based on the reduction of ferricytochrome c by O$_2^-$ to ferrocytochrome c, a reaction that can be detected by measuring the absorbance at 550 nm.

The electron spin resonance is the only method that is able to detect free radicals directly. This technique is based on the magnetic orientation and on the molecular environment of the unpaired electrons present in ROS.

**Indirect measurement of OS**

The indirect measurement of ROS in semen includes the following methods: 1) measurement of lipid peroxidation levels; 2) determination of the total antioxidant capacity (TAC) (enhanced-chemiluminescence or colorimetric); and 3) determination of the ROS-TAC score.
in seminal fluid and was validated by Sharma and collaborators.92 Contrary, the colorimetric evaluation of TAC is based on the antioxidant capacity of the sample to inhibit the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to ABTS+ by metmyoglobin. The values obtained are then compared with a standard, which is normally Trolox, a highly potent water-soluble vitamin E derivative as radical scavenger.93

Finally, the ROS-Tac score is a parameter based on the measurement of ROS and TAC. This score was created in order to provide a measure derived from the levels of ROS (oxidants) produced and the antioxidant levels in a sample and is therefore a measure of the balance between oxidants and antioxidants. It minimizes the variability from individual parameters of OS.92

**Measurement of oxidation reduction potential**

Oxidation reduction potential (ORP) was used over 50 years ago to determine if the oxidant activity was sufficiently high in treated water to kill bacteria and other microbes.94 ORP or the redox potential is a measure of the tendency of a compound A to acquire electrons from compound B whereby compound A will be reduced and compound B be oxidized. The greater the affinity for electrons, the higher the ORP of a redox pair. Hence, ORP is a reflection of the oxidative state of a chemical system, including cellular systems. Consequently, biological fluids, including semen also have an inherent ORP, which can be of clinical value as this is related to the status of biological and/or pathological processes. Thus, the ORP can provide information on the health status of a patient.95

Technically, ORP is a composite marker for an integrated
evaluation of the balance between total oxidants and antioxidants in a biological fluid and provides an overall oxidative status of the body fluid of the patient. However, the measurement techniques used to assess OS in a cellular system, such as semen, are based on single markers, which are not consistent. In addition, while most methods for the evaluation of OS in a cellular system are expensive, time consuming and require highly skilled technical expertise, the measurement of ORP is a simple and fast method to assess the overall oxidative status of semen.

Male infertility oxidative system (MiOXSYS®)

The development of the Male Infertility Oxidative System (MiOXSYS®) is an instrument that aims to overcome the negative aspects of other more complicated and expensive methods to evaluate OS in semen samples. MiOXSYS® is a galvanostat-based technique comprising the analyzer and a disposable sensor (Figure 2). It measures the redox potential in a rapid, simple and inexpensive way. The MiOXSYS® system provides the static ORP, which represents the actual redox balance in a given sample; higher ORP is indicative of oxidative stress. The advantages and disadvantages of the MiOXSYS® are summarized in Table I.

Protocol

MiOXSYS® is a simple system where a steady low voltage current is applied, and the activity of the electrons is measured in millivolts (mV). To measure the ORP in a semen or seminal plasma sample, after the analyzer is turned on, a MiOXSYS® Sensor is unwrapped (Figure 3) and placed on the port of the MiOXSYS® analyzer with the electrodes facing the MiOXSYS® Analyzer (Figure 4). Both, fresh or frozen semen or seminal plasma samples can be measured. Using a 30µL micropipette load of the sample on the application port taking care that no air bubbles are introduced, and the entire port is covered (Figure 5). Analysis will start once the sample reaches the reference cell of the sensor (Figure 2). It takes about 2 minutes for the sample to be

Table II.—Clinical studies in male fertility with ORP measurements by MiOXSYS®

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarwal86</td>
<td>Healthy male volunteers (N.=26)</td>
<td>MiOXSYS® measured ORP in semen and seminal plasma</td>
</tr>
<tr>
<td>Agarwal87</td>
<td>Proven fertile men (control) (N.=15)</td>
<td>ORP levels were higher in control group compared with NZ.</td>
</tr>
<tr>
<td>Agarwal87</td>
<td>In fertile men (Patient) (N.=293)</td>
<td>ORP levels were higher in patient group compared with NZ.</td>
</tr>
<tr>
<td></td>
<td>(The samples were categorized in differentiate controls NZ, OZ, AZ and TZ)</td>
<td>ORP has high predictive power for OZ patients</td>
</tr>
<tr>
<td></td>
<td>in USA:</td>
<td>A cut-off of 2.59 mV/10⁶ sperm from infertile men with OZ</td>
</tr>
<tr>
<td></td>
<td>• infertile patients (N.=194)</td>
<td>In USA, a cut-off of 1.42 mV/10⁶ sperm was able to differentiate between fertile and infertile (84.3% specificity and 49% sensitivity)</td>
</tr>
<tr>
<td></td>
<td>• fertile donors (N.=51)</td>
<td>In Qatar, a cut-off of 2.26 mV/10⁶ sperm allowed to differentiate between fertile and infertile (78% specificity and 60.8% sensitivity)</td>
</tr>
<tr>
<td></td>
<td>in Qatar:</td>
<td>Both centers, a cut-off of 1.42 mV/10⁶ sperm was able to differentiate between fertile and infertile men (74.3% specificity and 60.6% sensitivity)</td>
</tr>
<tr>
<td></td>
<td>• infertile patients (N.=400)</td>
<td>Proves the reproducibility and reliability in ORP measurements</td>
</tr>
<tr>
<td></td>
<td>• fertile donors (N.=50)</td>
<td>A cut-off of 1.57 mV/10⁶ sperm allowed to detect at least 1 abnormal parameter (88.1% specificity and 70.4% sensitivity)</td>
</tr>
<tr>
<td></td>
<td>in USA, a cut-off of 2.59 mV/10⁶ sperm allowed to detect OZ (91.2% specificity and 88% sensitivity)</td>
<td>Proves the reproducibility and reliability in ORP measurements</td>
</tr>
<tr>
<td>Agarwal86</td>
<td>Healthy donors (N.=49)</td>
<td>ORP levels were higher in samples with abnormal sperm parameters</td>
</tr>
<tr>
<td></td>
<td>Infertile patients (N.=194)</td>
<td>A cut-off of 1.57 mV/10⁶ sperm allowed to detect at least 1 abnormal parameter (88.1% specificity and 70.4% sensitivity)</td>
</tr>
<tr>
<td></td>
<td>• fertile donors (N.=50)</td>
<td>A cut-off of 2.59 mV/10⁶ sperm allowed to detect OZ (91.2% specificity and 88% sensitivity)</td>
</tr>
<tr>
<td>Majzoub97</td>
<td>Proven fertile men (N.=50)</td>
<td>In infertile men the ORP values were inversely related with total sperm count, motility and morphology</td>
</tr>
<tr>
<td></td>
<td>Infertile men (N.=365)</td>
<td>ORP values were higher in samples with abnormal quality compared with normal quality</td>
</tr>
<tr>
<td></td>
<td>• Proven fertile men (N.=100)</td>
<td>Infertile patients presented higher values of ORP when compared with fertile men</td>
</tr>
<tr>
<td></td>
<td>• Infertile men (N.=1168)</td>
<td>A cut-off of 1.38 mV/10⁶ sperm allowed to differentiate normal from abnormal samples (87.8% specificity and 63.3% sensitivity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A cut-off of 1.41 mV/10⁶ sperm allowed to differentiate fertile from infertile men (78% specificity and 57.3% sensitivity)</td>
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<tr>
<td></td>
<td></td>
<td>Proves the reproducibility and reliability in ORP measurements</td>
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</table>

AZ: asthenozoospermic; NZ: normozoospermic; ORP: oxidation-reduction potential; OZ: oligozoospermic; TZ: teratozoospermic.
Clinical relevance of OS in male fertility

The negative effect of OS on sperm quality and consequently the male fertility potential has repeatedly been described. The role of ROS in sperm was discovered long time ago. While physiological levels of ROS are necessary for normal physiological function of spermatozoa, excessive ROS will have detrimental effects. The subject is not new, but the role of ROS in spermatozoa is still a matter of debate in male fertility; the oxidative status of the spermatozoa or semen directly. Furthermore, the available methods are time consuming, laborious and require expensive equipment and a trained operator. In contrast, the evaluation of the oxidative state of a semen sample using the MiOXSYS® system is cheap, timesaving, reproducible and easy. This system evaluates seminal oxidative stress in simple, fast and inexpensive way. Combinated with the MiOXSYS® system, it is an attractive alternative for the evaluation of the oxidative state of a sample in an andrological laboratory setting. ORP is not only able to distinguish normal and abnormal semen samples but is also able to differentiate fertile from infertile men.

Conclusions

The basic semen analysis remains the “cornerstone” in male fertility evaluation. However, it has a limited predictive value for fertilization to occur. Oxidative stress is implicated in the etiology of male infertility. The role of ROS in sperm was discovered long time ago. While physiological levels of ROS are necessary for normal physiological function of spermatozoa, excessive ROS will have detrimental effects. The subject is not new, but the role of ROS in spermatozoa is still a matter of debate in male fertility; the oxidative status of the spermatozoa or semen directly. Furthermore, the available methods are time consuming, require expensive equipment and a trained operator. In contrast, the evaluation of the oxidative state of a semen sample using the MiOXSYS® system is cheap, timesaving, reproducible and easy. This system evaluates seminal oxidative stress in simple, fast and inexpensive way. Combinated with the MiOXSYS® system, it is an attractive alternative for the evaluation of the oxidative state of a sample in an andrological laboratory setting. ORP is not only able to distinguish normal and abnormal semen samples but is also able to differentiate fertile from infertile men.

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