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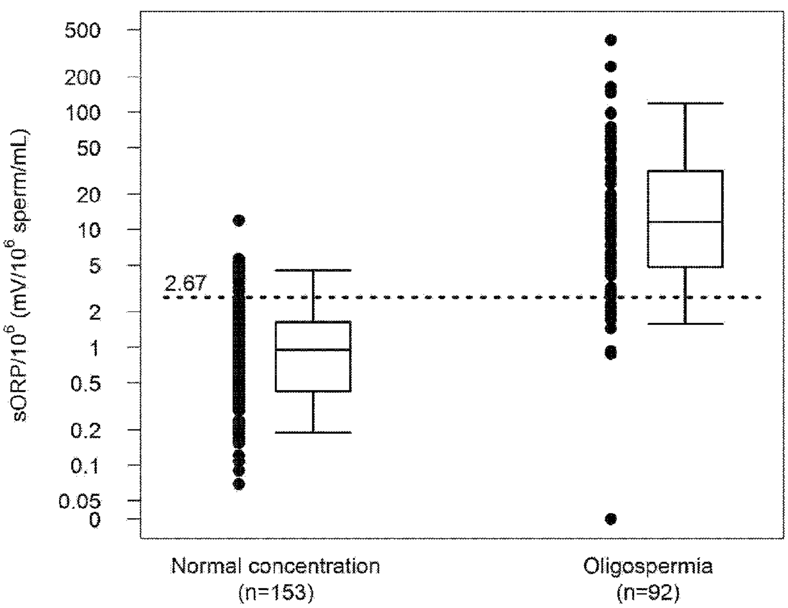

Figure 6

(57) **Abstract:** Methods for treatment of infertility and for determining appropriate treatment of infertility in varicocele patients involving the use of the oxidation-reduction potential (ORP) status of semen samples are provided. In addition, methods for determining the effectiveness of a varicocelectomy procedure on infertile male subjects is provided.

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METHODS FOR TREATMENT OF INFERTILITY IN VARICOCELE PATIENTS

FIELD

The present invention relates to methods for treatment of infertility in varicocele patients involving the use of the oxidation-reduction potential (ORP) status of semen samples.

BACKGROUND

When the pampiniform plexus veins in the scrotum become enlarged, the resulting enlarged veins are known as varicoceles. Varicoceles are similar to varicose veins in the leg. Varicoceles form during puberty and can grow larger and easier to notice over time. Varicoceles are more common on the left side of the scrotum, but can exist on both sides at the same time, although this is rare. Varicoceles occur in about 10-15% of males. Most of the time, varicoceles cause no problems and are harmless. Less often varicoceles can cause pain, infertility, or slow growth or shrinkage of a testicle.

The presence of a varicocele can be confirmed with ultrasound, which can show dilation of the vessels of the pampiniform plexus to be greater than 2 mm. Varicoceles are classified by grade, with grade 1 being the smallest and not visible, but can be felt by a physician using a Valsalva maneuver. Grade 2 is also not visible, but can be felt without a Valsalva maneuver, and grade 3 is visible.

One concern of varicocele is its potential impact on male infertility, although the relationship between varicocele and infertility is unclear. Men with varicocele can be fertile or infertile and can have sperm with normal structure and function or can have sperm with abnormal structure and/or function. However, men with varicocele tend to have poor sperm quality and are at higher risk of being infertile. One option for treatment of varicocele is a varicocelectomy, in which the area is accessed surgically and using ultrasound and surgical microscopes, the affected veins are closed to reroute blood through other, healthier vessels.

The pathophysiology of infertility in men with varicocele has been studied but the underlying mechanism is not clear. One of the pathophysiologic mechanisms for infertility in these patients proposed is oxidative stress. Patients with clinical varicocele have been found to have elevated static oxidation reduction potential (sORP) in semen samples, with the highest levels of sORP in grade 3 varicocele patients.

There remains a need for applications of the oxidative stress status of patients with varicocele in treating infertility.

SUMMARY

One embodiment of the invention is a method of treating infertility in a male subject with varicocele that includes measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject. The measured sORP is compared to a reference value, and if the measured sORP value is an elevated sORP as determined by the comparison, the method further includes administering a therapy to improve mitochondrial function. Impaired mitochondrial function in infertile men with varicocele leads to oxidative stress and treatment of the underlying dysfunction can improve the fertility status of the subject.

The therapy to improve mitochondrial function can include administering an antioxidant to the subject. For example, such therapy can include administering an antioxidant selected from L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, vitamin B12, zinc, alpha lipoic acid, B-vitamins; fish or krill oil, glutathione, magnesium, nicotinamide, riboside; pyrroloquinoline quinone and combinations thereof to the subject. Alternatively, the therapy can include one or more lifestyle activities to improve mitochondrial function, such as increased movement (high intensity interval training and strength training), improved sleep, reducing stress, sun exposure and exposure to cold temperatures.

In this embodiment, an elevated sORP can be an sORP of 2.30 mV/10^6 sperm/mL or greater up to 3.2 mV/10^6 sperm/mL or greater. In one embodiment, an elevated sORP can be an sORP of 2.67 mV/10^6 sperm/mL or greater.

In this embodiment, the subject infertile male subject can have clinical grade 2 or 3 varicocele or can have clinical grade 3 varicocele.

In this embodiment, the step of measuring the sORP can include first liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.

Another embodiment of the invention is a method of determining a treatment for a male subject having infertility. The method includes evaluating the subject to determine the presence of a varicocele in the subject, and if the subject has a varicocele, measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject. The measured value is compared to a reference value and if it is elevated sORP as determined

by the comparison, performing a varicocelectomy on the subject to improve fertility of the subject.

In this embodiment, an elevated sORP can be an sORP of 2.30 mV/10^6 sperm/mL or greater up to 3.2 mV/10^6 sperm/mL or greater. In one embodiment, an elevated sORP can be an sORP of 2.67 mV/10^6 sperm/mL or greater.

In this embodiment, the subject infertile male subject can have clinical grade 2 or 3 varicocele or can have clinical grade 3 varicocele.

In this embodiment, the step of measuring the sORP can include first liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.

A further embodiment of the invention is a method of determining the effectiveness of a varicocelectomy procedure on an infertile male subject at improving fertility. The method includes measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject prior to the procedure and measuring the sORP of a semen sample from the subject after the procedure. If the sORP of the semen sample after the procedure is less than the sORP of the semen sample before the procedure, the procedure was effective at improving the fertility of the subject. The sample taken after the procedure can be taken at a time period between about 30 days post-procedure to about 180 days post-procedure and in one embodiment, can be taken at about 90 days post-procedure.

In this embodiment, an elevated sORP can be an sORP of 2.30 mV/10^6 sperm/mL or greater up to 3.2 mV/10^6 sperm/mL or greater. In one embodiment, an elevated sORP can be an sORP of 2.67 mV/10^6 sperm/mL or greater.

In this embodiment, the subject infertile male subject can have clinical grade 2 or 3 varicocele or can have clinical grade 3 varicocele.

In this embodiment, the step of measuring the sORP can include first liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a comparison between the sORP values measured from samples tested as semen (X axis) and again as seminal plasma (Y axis), based on sORP/Concentration (mV/ 10^6 sperm / mL).

Fig. 2 shows a comparison between the raw sORP values (mV) of semen and seminal plasma.

Fig. 3 illustrates the effect of freezing the sample on the sORP values of semen and seminal fluid.

Fig. 4 illustrates the effect of time on sORP/Conc. value of semen (10A) and seminal plasma (10B). X axis is the sORP Conc. value of semen (10A) or seminal plasma (10B) at 0 minutes. Y axis is the sORP/Conc. value of semen (10A) or seminal plasma (10B) at 120 minutes.

Fig. 5 illustrates the effect of time on sORP value (mV) of semen (10A) and seminal plasma (10B). Left bar in each graph is the sORP value (mV) at 0 minutes. Right bar in each graph is the sORP value (mV) at 120 minutes.

Fig. 6 illustrates a comparison between the sORP value (mV) of semen samples from men with normal sperm counts and from oligospermic men.

DETAILED DESCRIPTION

Embodiments of the present invention provide methods for treatment of infertility in varicocele patients involving the use of the oxidation-reduction potential (ORP) status of semen samples. Devices and methods for measuring oxidation-reduction potential (ORP) characteristics (*i.e.*, static oxidation-reduction potential (sORP) and/or oxidation-reduction capacity (cORP)) of a fluid that are suitable for rapid, routine clinical diagnostic testing are known and disclosed in, for example, U.S. Patent Nos. 8,317,997; 9,360,446; 9,372,167; and 10,088,466, all of which are hereby incorporated by reference in their entirety.

As can be appreciated by one of skill in the art after consideration of the present disclosure, the measurement of the sORP value can be in units of Volts, and the integration of the current signal therefore gives a value representing a quantity of charge in Coulombs. cORP values, as a measure of a quantity of charge, is expressed herein as one over the quantity of charge in Coulombs. In particular, by taking the inverse of the observed quantity of charge, a more normal distribution is obtained, facilitating the application of parametric statistics to observed ORP values. As used herein, the terms ORP capacity, inverse capacity levels, inverse capacity ORP or ICL are all equivalent to cORP as defined above. It will be appreciated that expression of cORP as one over a quantity of charge encompasses alternative equivalent expressions.

As noted above, higher than normal values of sORP are indicative of oxidative stress and are generally considered to be a negative indication for the subject being evaluated. cORP is a measure of a subject's capacity to withstand oxidative insult. Thus, it is generally a positive indication for a subject to have a normal or higher capacity to withstand oxidative insult. Since cORP is defined as the inverse of the quantity of charge to reach a voltage inflection point, a higher cORP value is indicative of a lesser capacity to withstand oxidative insult, and likewise, a lower cORP value is indicative of a greater capacity to withstand oxidative insult.

The present invention includes embodiments for monitoring or treating infertile male patients having varicocele. One embodiment of the invention is method of treating infertility in a male subject with varicocele that includes measuring the sORP of a semen sample from the subject and comparing the measured sORP to a reference value. If the measured sORP value is an elevated sORP as determined by comparison to the reference value, the method further includes administering a therapy to the subject to improve mitochondrial function. As shown below in Examples 9 and 10, impaired mitochondrial function in infertile men with varicocele leads to oxidative stress and sperm dysfunction and varicocelectomy improves oxidation reduction potential in infertile men. Another embodiment of the invention is a method of determining treatment for a male subject having infertility that includes evaluating the subject to determine the presence of a varicocele in the subject. If the subject has a varicocele, measuring the sORP of a semen sample from the subject and comparing the measured sORP to a reference value. If the measured sORP value is elevated sORP as determined by comparison to the reference value, the method further includes performing a varicocelectomy on the subject to improve fertility of the subject. A further embodiment of the invention is a method of determining the effectiveness of a varicocelectomy procedure on an infertile male subject at improving fertility. This method includes measuring the sORP of a semen sample from the subject prior to the procedure and after the procedure. If the sORP of the semen sample after the procedure is less than the sORP of the semen sample before the procedure (or less than a reference value as discussed below), the procedure was effective at improving the fertility of the subject.

The foregoing statement of embodiments of the invention and other descriptions herein refer to measurement of sORP. Still further embodiments of the invention include the use of measurements of cORP as an alternative to or in addition to sORP in the various embodiments. Thus, in one embodiment of the invention is method of treating infertility in a male subject with varicocele that includes measuring the cORP and/or sORP of a semen sample from the subject and comparing the measured cORP and/or sORP to a reference value. If the measured value is an elevated as determined by comparison to the reference value, the method further includes administering a therapy to the subject to improve mitochondrial function. Another embodiment of the invention is a method of determining treatment for a male subject having infertility that includes evaluating the subject to determine the presence of a varicocele in the subject. If the subject has a varicocele, measuring the cORP and/or sORP of a semen sample from the subject and comparing the measured value to a reference value. If the measured value is elevated as determined by comparison to the reference value, the method further includes performing a varicocelectomy on the subject to improve fertility of the subject. A further embodiment of the invention is a method of determining the effectiveness of a varicocelectomy procedure on an infertile male subject at improving fertility. This method includes measuring the cORP and/or sORP of a semen sample from the subject prior to the procedure and after the procedure. If the cORP and/or sORP of the semen sample after the procedure is less than the cORP and/or sORP of the semen sample before the procedure, the procedure was effective at improving the fertility of the subject.

As used herein, reference to male patients or subjects who are "infertile" refer to both subjects who are either sub-fertile or infertile, unless specifically noted otherwise. As used herein, sub-fertile refers to an individual having a condition making conception less likely, but not impossible. Thus, sub-fertility refers to any form of reduced fertility having a prolonged time of unwanted non-conception. For example, as used herein, the term sub-fertile refers to an individual who is unable to achieve a successful pregnancy after 6 months, or 7 months, or 8 months, or 9 months, or 10 months, or 11 months of unprotected intercourse. For example, as used herein, the term infertile refers to an individual who is unable to achieve a successful pregnancy after 12 months of unprotected intercourse. Such terminology is understood by those in the field of reproductive medicine.

It will be understood by those in the field that the terms sub-fertile and infertile are clinical definitions based on time to conception. Consequently, such diagnoses may be different points on a relative scale. For example, an individual, or couple, diagnosed as sub-fertile may later be diagnosed as infertile. Alternatively, an individual, or couple, diagnosed as infertile may later be found to be sub-fertile.

Various embodiments of the invention involve treating, determining the treatment of, or determining the effectiveness of a varicocelectomy procedure on men with varicocele. The presence of a varicocele in a male subject is a clinically determined condition When the pampiniform plexus veins in the scrotum become enlarged, the resulting enlarged veins are known as varicoceles. Varicoceles are similar to varicose veins in the leg. Varicoceles form during puberty and can grow larger and easier to notice over time. Varicoceles are more common on the left side of the scrotum, but can exist on both sides at the same time, although this is rare. Varicoceles occur in about 10-15% of males. Most of the time, varicoceles cause no problems and are harmless. Less often varicoceles can cause pain, infertility, or slow growth or shrinkage of a testicle.

The presence of a varicocele can be confirmed with ultrasound, which can show dilation of the vessels of the pampiniform plexus to be greater than 2 mm. Varicoceles are classified by grade, with grade 1 being the smallest and not visible, but can be felt by a physician using a Valsalva maneuver. Grade 2 is also not visible, but can be felt without a Valsalva maneuver, and grade 3 is visible.

The embodiments of the invention include the measurement of the ORP of a semen sample. In some embodiments, measurement of the ORP of a semen can include liquefying the semen sample and then measuring the ORP (sORP and/or cORP) normalized as a sperm cell concentration value. The measurement can be done within 120 minutes of liquefying the semen sample.

In various embodiments of the invention, a measured ORP is compared to a reference ORP. Typically, the ORP characteristics of the subject are compared to an ORP characteristic reference value or values that are relevant to that subject. As used herein, a reference value can be an ORP characteristic of the patient from a time when the patient did not have the condition in question (*e.g.*, when he was fertile) or from an earlier time period when the patient had the condition in question (for purposes of monitoring or evaluating the condition or treatment thereof). Such reference values are referred to as self reference values. For example, reference values can also include initial, maximum and ending reference values, such as when ORP characteristics are evaluated over a time frame such as when a patient was known to be fertile (initial), and at a time when a patient is known to be sub-fertile or infertile (ending). Alternatively, a reference value can be an ORP characteristic of a relevant healthy population (*e.g.*, a fertile population that is matched in one or more characteristics of species, age, sex, ethnicity, etc.). Such reference values are referred to as normal reference values. Further, a reference value can be an ORP characteristic of a relevant population similarly situated as the patient (*e.g.*, a population having the same or similar condition (e.g., sub-fertility or infertility) as the patient for which the patient is being treated and preferably, one that is also matched in one or more characteristics of species, age, sex, ethnicity, etc.). Such a reference value is referred to as a condition specific reference value. For example, a condition specific reference value can be an ORP value obtained from a sub-fertile or infertile individual, an incompetent egg or a sperm sample having low fertility potential.

As described above, a reference value for a measured ORP (sORP and/or cORP) of a semen sample in the various embodiments of the invention can be self reference values, normal reference values, or condition specific reference values. In some embodiments, a reference value or cut-point for identification of elevated ORP values can be ORP values that are 2.30 mV/10^6 sperm/mL or greater, 2.35 mV/10^6 sperm/mL or greater, 2.40 mV/10^6 sperm/mL or greater, 2.45 mV/10^6 sperm/mL or greater, 2.50 mV/10^6 sperm/mL or greater, 2.55 mV/10^6 sperm/mL or greater, 2.60 mV/10^6 sperm/mL or greater, 2.65 mV/10^6 sperm/mL or greater, 2.70 mV/10^6 sperm/mL or greater, 2.75 mV/10^6 sperm/mL or greater, 2.80 mV/10^6 sperm/mL or greater, 2.85 mV/10^6 sperm/mL or greater, 2.90 mV/10^6 sperm/mL or greater, or 2.95 mV/10^6 sperm/mL or greater. Alternatively, a reference value or cut-point for identification of elevated ORP values can be ORP values that are 2.60 mV/10^6 sperm/mL or greater, 2.61 mV/10^6 sperm/mL or greater, 2.62 mV/10^6 sperm/mL or greater, 2.63 mV/10^6 sperm/mL or greater, 2.64 mV/10^6 sperm/mL or greater, 2.65 mV/10^6 sperm/mL or greater, 2.66 mV/10^6 sperm/mL or greater, 2.67 mV/10^6 sperm/mL or greater, 2.68 mV/10^6 sperm/mL or greater, 2.69 mV/10^6 sperm/mL or greater, 2.70 mV/10^6 sperm/mL or greater, 2.71 mV/10^6 sperm/mL or greater, 2.72 mV/10^6 sperm/mL or greater, or 2.73 mV/10^6 sperm/mL or greater. Alternatively, a reference value or cut-point for identification of elevated ORP values can be an ORP value that is any 1/100th of a mV/10^6 sperm/mL or greater value between 2.30 and 3.20.

As used herein, a subject is any individual for whom a biological sample is being tested for an ORP characteristic. The term subject can include a patient if the subject is an individual being treated by a medical professional. The terms subject and patient can refer to any male animal, including humans and non-human animals, such as companion animals (e.g., cats, dogs, horses, etc.) and livestock animals (i.e., animals kept for food purposes such as cows, goats, chickens, etc.). Preferred subjects include mammals and most preferably include humans.

As used herein, the terms sperm and spermatozoa can be used interchangeably to refer to a male reproductive cell. According to the present disclosure, the term semen, semen sample, and the like, have the standard meaning used in the art. That is, semen is male reproductive fluid comprising spermatozoa and fluid from the seminal vesicles and fluid from the prostate gland and other reproductive glands.

In various embodiments of the invention, the ORP characteristics of a biological sample of a subject are measured. The measurement of the ORP characteristics of a biological sample can be done at one or at multiple time points. The frequency of such measurements will depend on the condition being evaluated. For example, evaluation of the fertility of a male subject may require a single test.

In the embodiment of the invention of treating infertility in a male subject with varicocele by measuring the sORP of a semen sample from the subject and if elevated, administering a therapy to the subject to improve mitochondrial function, the therapy for improving mitochondrial function can include any of various known compounds and composition for improving mitochondrial function. For example, the therapy can include administering an antioxidant compound or composition to the subject. More specifically, the antioxidant composition can include a compound selected from L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, vitamin B12, zinc, alpha lipoic acid, B-vitamins; fish or krill oil, glutathione, magnesium, nicotinamide, riboside; pyrroloquinoline quinone (PQQ) and combinations thereof. In addition, a variety of lifestyle activities are known to improve mitochondrial function, such as increased movement (high intensity interval training and strength training), improved sleep, reducing stress, sun exposure and exposure to cold temperatures.

In the embodiments of the invention that involve determining the effectiveness of a varicocelectomy procedure on infertile men with varicocele, the sORP of a semen sample is measured after the procedure is conducted. Such post-procedure samples can be taken at a time period between about 30 days post-procedure to about 180 days post-procedure or any range within the 30-180 day post-procedure range, such as for example, 30-40 days, 80-100 days, or 115-125 days post-procedure.

In various embodiments of the invention, in addition to evaluation of ORP as a criteria, for example, for administering therapy to improve mitochondrial function, or for performing a varicoceletomy procedure, the methods of the invention can include consideration of the grade of varicocele of the subject. Thus, the methods can include a limitation that the subject has a grade 2 or a grade 3 varicocele, in addition to an elevated ORP value, before administering any treatment or that the subject has a grade 3 varicocele before administering any treatment.

In other embodiments of the invention, in addition to evaluation of ORP as a criteria, for example, for administering therapy to improve mitochondrial function, or for performing a varicoceletomy procedure, the methods of the invention can include consideration of more traditional semen parameters, including sperm count, total motility, progressive motility, and morphology. These parameters can be based on accepted clinical standards for normal and abnormal classification, such as, the guidelines in the WHO laboratory manual for the examination and processing of human semen, 5^{th} edition 2010. Thus, the methods can include a limitation that the subject has one, two, three, four or more semen criteria that are outside of a normal classification, in addition to an elevated ORP value, before administering any treatment.

In order to determine the trend of ORP characteristics in an individual over time, without limitation, the ORP characteristics of the individual may be checked at suitable intervals, limited only by the physical restraints of generating a biological sample. For example, ORP characteristics can be checked every day, 2 days, 3 days, 4 days, 5 days, 6 days, week, 2 weeks, 3 weeks, month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or year.

In a related embodiment, the semen sample being analyzed for its fertility potential can be a stored sample. Such samples are well known in the field of reproductive biology. Moreover, it is appreciated that spermatozoa are usually cryogenically preserved and that such storage involves the use of cryoprotectant agents, such as glycerol and dimethyl sulfoxide. Recent evidence suggests that different cryoprotectants have different effects on the immediate environment of the stored spermatazoa and that the storage environment can affect the fertility potential of the stored sample. Thus, in one embodiment the ORP characteristics of a stored sample of spermatozoa are determined in order to assess the fertility potential of the sperm in the sample. The sample can be a semen sample or it can be a semen sample that has been further processed (e.g., centrifugation, washing, addition of buffers, cryoprotective agents, antioxidants, and the like) prior to storage. In one embodiment, the ORP characteristics are used to measure the effect of various storage conditions on sperm viability and/or fertility potential. In certain embodiments, the storage conditions are varied by the addition of antioxidants, such as for example, ascorbic acid and α-tocopherol.

This PCT application claims the benefit of priority to U.S. Provisional Patent application no. 62/579,725 filed on October 31, 2017, the disclosure of which is hereby incorporated by reference in its entirety.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the embodiments, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for.

Example 1. Establishment of Oxidation-Reduction Potential in Semen and Seminal Plasma

This Example demonstrates that ORP can be measured in semen and seminal plasma samples.

Male partners from couples concerned about infertility were recruited from a single international andrology laboratory (Clinical Studies) or from a national urology laboratory (Technical Studies). A single semen sample was donated by each participant. Semen samples were allowed to liquefy at room temperature for approximately 30 minutes, following WHO guidelines (5th Edition, 2010) for liquefaction. Following liquefaction, the samples were divided into two fractions. One fraction was centrifuged at 300g for seven minutes to isolate the seminal plasma.

Semen Analysis

Following liquefaction, ejaculate volume, sperm cell number, sperm concentration, morphology, total and progressive motility were measured using WHO guidelines for sperm quality (2010, 5th Edition). Smears were stained with a Diff-Quik kit for assessment of sperm morphology. Samples were also tested for Leukocytospermia, i.e. >1 X 10^6 WBC/mL when the round cell concentration was >1 X10^6 and confirmed by the peroxidase or the Endtz test. Sperm count and motility were assessed manually.

Oxidation-Reduction (ORP) Measurement

sORP (mV/10^6 sperm/mL) was measured in both fractions using the RedoxSYS test comprised of a Redox SYS analyzer MiOXSYS (Aytu BioScience) sensor (MiOXSYS, Aytu BioScience). The sensor was inserted face-up and with the sensor electrodes facing the MiOXSYS Analyzer. Using a micropipette, 30μL of sample (semen or seminal plasma) was released onto the sensor's application port. Once the sample reached the reference cell of the sensor, the testing automatically began and audible beeps indicated completion of the test. The sORP (in millivolts or mV) was recorded. Analysis was performed in triplicate and the average values were corrected for sperm concentrations and expressed as sORP (mV/10^6 sperm). Values are reported as mean ±

SEM. Spearman correlation and Receiver Operating Characteristic curves (ROC) were used for statistical analysis.

Statistical Analysis

Spearman correlation test was used for statistical analysis to compare qualitative variables. A P value of < 0.05 was considered statistically significant.

The results of these analyses are shown in Figures 1 and 2. The results demonstrate that the MiOXSYS test can accurately measure ORP in semen and seminal plasma levels, and that the sORP values semen matched those measured in seminal plasma.

Example 2. Comparison of sORP/Concentration Measures Using Fresh and Frozen Samples

Semen samples were obtained and treated as described in Example 1. Samples were then stored at -80ºC for 120 minutes, after which they were thawed, brought to room temperature and sORP measured. The result of this analysis is shown in Figure 3.

The results demonstrate freezing samples, either as semen or seminal plasma, does not alter the sORP value, and further confirms the lack of difference between semen and seminal plasma samples.

Example 3. Effect of Time on Oxidation-Reduction Potential in Semen and Seminal Plasma

Semen samples were obtained and treated as described in Example 1. sORP was measured immediately after liquefaction (0 minutes). The samples were then left at room temperature for 120 minutes and retested (120 minutes). The results are shown in Figures 4 and 5.

The results demonstrate that there is no difference in sORP values in samples that were tested immediately and those which were measured at a later time point.

Example 4. Multi-center evaluation of oxidation reduction potential assay in the infertile male.

OBJECTIVE: Evaluate if oxidation reduction potential (ORP) levels using the MiOXSYS analyzer could differentiate semen samples that meet the normal reference range of WHO criteria from those that do not use samples from multiple centers.

DESIGN: This study was carried out at 8 different institutions across the world. The study was approved by the Ethics committee of each participating institution (Cleveland Clinic, Cleveland, USA; Hamad Medical Center, Doha, Qatar; Dokkyo University, Osaka, Japan; The Doctor's Lab-oratory, London, UK; VKF American Hospital, Istanbul, Turkey; Sohag

University, Sohag, Egypt; Bezmi Alem Vakif University, Istanbul, Turkey; Assam University, Silchar, India; Tulane Medical Center, New Orleans, USA) and all subjects consented prior to participation. Patients (n = 2010) were grouped into those that had all normal semen parameters (concentration, total motility, and morphology) according to WHO 2010 guide-lines and those who failed to meet one or more criteria.

MATERIALS AND METHODS: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of prescription, OTC medications or antioxidants. Semen parameters were assessed using the WHO fifth edition guidelines (2010). ORP was measured (mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration $>0.999x10^6$ sperm/mL were included.

RESULTS: 1804 samples had at least one abnormal sperm parameters according to WHO criteria; 206 samples had sperm parameters that fell within the normal range. ORP results were negatively correlated with sperm concentration ($p<0.01$), total sperm ($p<0.01$) progressive motility ($p<0.01$, total motility ($p<0.01$, morphology ($p<0.01$). The area under the curve (AUC) was 0.757. An ORP cut-off value of 1.34 mV/10^6 sperm/mL was able to differentiate samples with abnormal semen parameters with 58% sensitivity, 85% specificity, with positive predictive value of 96 and a negative predictive value of 42.

CONCLUSIONS: ORP levels can serve as an adjunct to routine semen analysis. Abnormal ORP levels will be especially useful in pinpointing the altered functional status of the sperm in patients with idiopathic male infertility and thereby directing those men to accurate therapeutic management.

Example 5. Oxidation reduction potential: a reliable and reproducible method.

OBJECTIVE: Seminal oxidative stress (OS) is well known to affect male fertility status. The lack of reproducibility in OS measurement has hindered its clinical use as a quality indicator for semen. Some tests measure single markers of oxidants or reductants, leading to lack of standardization of results. Oxidation reduction potential (ORP) can better measure OS as it provides an overall measure of the activity of both oxidants and reductants. The goals of this multicenter study was to investigate 1) the reproducibility and reliability of the ORP measurement as an indicator for sperm quality across different fertility centers and 2) establish the ORP cutoff value to distinguish infertile men from healthy controls.

DESIGN: ORP measurement in infertile men with abnormal sperm parameters and healthy control in two fertility centers.

MATERIALS AND METHODS: Semen analysis and ORP measurements from two andrology laboratories in the USA and Qatar over a period of 12 months was collected. The USA dataset contained 194 patients and 51 fertile donors, while the Qatar dataset contained 400 patients and 50 fertile donors. Semen analyses were performed followed by ORP measurements using the MiOXSYS analyzer. Semen samples with abnormal sperm parameters were identified based on the WHO 5th edition guidelines. The ORP and abnormal sperm parameters were compared using Student's t-test and a P value of <0.05 was considered significant. The area under the curve for different diagnostic predictive values of ORP in these groups were calculated with receiver operating characteristic (ROC) analysis.

RESULTS: In the USA, Qatar, and combined datasets, the infertile group had significantly lower sperm concentration, total and progressive motility, and normal morphology as well as higher ORP levels compared to fertile men (P< 0.05). When comparing data from both centers, the infertile group showed significant difference between both datasets regarding progressive motility and morphology (P< 0.001). The percentage of patients with abnormal semen volume, sperm count, total and progressive motility were significantly different between the two male infertility centers (P< 0.05). ORP levels showed no significant difference between both data sets (P<0.08). ROC analysis indicated that ORP cut-off value of 1.42 mV/106/mL dataset can accurately differentiate fertile from infertile semen groups. CONCLUSIONS: The measurements for ORP among infertile men were consistent between centers in the USA and Qatar and with previous studies. ORP remains stable even with measurable differences in other sperm parameters, and it therefore can be used in addition to semen analysis to confirm poor semen quality or as a possible independent diagnostic tool for assessing infertility. Overall, ORP is a reliable method of measuring OS and can be used by laboratories worldwide as a standard part of assessing semen quality.

Example 6. Relationship between seminal oxidation reduction potential and sperm DNA fragmentation in infertile men.

OBJECTIVE: Oxidation reduction potential (ORP) is a new tool to measure oxidative stress (OS) and has been shown to serve as an accurate predictor of poor semen quality in infertile men. Recent studies have shown that ORP test is a simple, quick, inexpensive and reproducible test of OS status in semen. Assessment of sperm DNA fragmentation (SDF) has been shown recently to correlate with fertility outcome in spontaneous pregnancy and assisted reproduction. OS is known as a major cause of SDF. We therefore, set out to investigate the relationship between the ORP and SDF in patients with male infertility.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Study included 309 infertile patients (Group 1) and 47 normal fertile donors (controls; Group 2) between Jan to Jun 2016 at a tertiary medical center. Patients with azoospermia, leukocytospermia, history of smoking, sexually transmitted diseases or those receiving antioxidants were excluded. Data on medical history, physical examination, semen analysis, ORP and SDF testing was collected.

RESULTS: The mean age of all subjects was 35.6 ± 7.85; and there were no differences between the two groups. Patients had significantly higher ORP (2.74 ± 3.92 vs. 1.26 ± 1.12 mV/10^6 sperm/mL; P<0.001) and SDF (27.6 ± 17.8% vs. 15.68 ± 6.31%, P<0.001) than controls. ORP levels correlated significantly with SDF in all subjects (r = 0.256; P<0.001) and in patients group (r = 0.222; P<0.001).

CONCLUSIONS: Correlation of ORP levels with SDF confirms the causal relationship between OS and SDF. ORP could be used as a surrogate marker for SDF in clinics which lack access to highly complex sperm function testing due to the expense or need for highly trained laboratory personnel.

<u>Example 7</u>. High seminal oxidation reduction potential in cryopreserved semen from infertile men is a marker of poor post-thaw sperm quality.

OBJECTIVE: Cryopreservation causes deleterious effects on human spermatozoa due to freezing and thawing, leading to decreased cryosurvival rates (CSR). The objectives of this study were to assess levels of oxidation-reduction potential (ORP) in cryopreserved semen of infertile men, and to determine their relationship to post-thaw sperm parameters.

DESIGN: A prospective cohort study.

MATERIALS AND METHODS: The study included 28 semen samples obtained from men who were evaluated for an infertility problem between November, 2016 and April, 2017. Standard semen analysis was performed according to the WHO guidelines (fifth edition, 2010). Fresh seminal ORP was measured using the MiOXSYS system (Aytu BioScience, Inc., Engle-wood, CO, USA). Recorded ORP values were adjusted for sperm concentration and final results were expressed as mv/106 sperm/ml. Semen samples with azoospermia, sperm concentration < 1 million/ml or leukocytospermia were excluded. Aliquots of 0.5 ml semen were cryopreserved using slow freezing technique. One week later, frozen samples were thawed at 37 °C, and examined for post-thaw percent of total motility, percent of progressive motility, total motile sperm (TMS) counts and ORP levels.

Cryosurvival rate was calculated according to the equation: CSR = post-thaw TMS/pre-freeze TMS X 100. Data were presented as median (25^{th} and 75^{th} percentiles). Paired sample t test was used for comparison of the pre and post-thaw results. P value < 0.05 was considered significant.

RESULTS: Post-thaw percent of total motility [20 (10, 40)], percent of progressive motility [10 (5, 25)] and TMS counts [4.1 (0.6, 6.3) X10^6 sperm] were significantly lower than pre-freeze values (percent of total motility [50 (40, 55)], percent of progressive motility [30 (24, 35)] and TMS counts [25 (18, 41) X106 sperm); P values < 0.001. Post-thaw levels of seminal ORP [2.8 (2.3, 4.4) mv/106 sperm/ml] were significantly higher than pre-freeze values [0.9 (0.54, 1.34) mv/10^6 sperm/ml]; P < 0.001. The median percentage of CSR was 10 (5 & 20). A significant (P< 0.05) negative correlation was found between post-thaw levels of seminal ORP and total motility (r = -0.5), progressive motility (r = -0.41), TMS counts (r = -0.60) and CSR (r = -0.52).

CONCLUSIONS: Sperm cryopreservation in infertile men was associated with high seminal ORP, low sperm motility and reduced CSR. Sperm cryo-damage is related to high seminal ORP generated during freeze-thaw process. Reduction in oxidant production will improve sperm recovery following cryopreservation.

Example 8. Oxidation-reduction potential: a new marker of oxidative stress and sperm quality.

OBJECTIVE: A negative association exists between the poor semen parameters and oxidative stress. Oxidation-Reduction Potential (ORP) is re-ported as an easy and effective measure of oxidative stress; while the value of routine semen analysis in predicting fertility remains highly contested due to great amount of variability in semen parameters and subjective nature of assessment. The objective of this example was to evaluate if ORP could serve as an adjunct to routine semen analysis in accurate prediction of semen quality.

DESIGN: Routine semen analysis and the ORP measurement in infertile men.

MATERIALS AND METHODS: Sperm parameters were evaluated in 84 healthy donors and 301 infertile men according to WHO 5th edition guide-line. ORP was measured using the MiOXSYS analyzer. ROC curve was generated and a cutoff identified. Semen parameters were compared in infer-tile men with low ORP and high ORP.

RESULTS: We identified a low ORP cutoff value of ≤1.30 mVolts/ 10^6 sperm/mL and >5.14 mVolts/10^6 sperm/mL as high ORP cutoff. A notably higher percentage of patients were above the ORP cutoff than the donors. Significantly poorer semen parameters were seen in patients with high ORP >5.14 when compared to those with low ORP <5.14 (p <0.001) (Table 1).

CONCLUSIONS: There is a direct relationship between poor semen quality and high ORP. The ORP levels can serve not only as an accurate measure of oxidative stress but has the potential to increase the reliability of routine semen analysis in the prediction of sperm quality.

Table 1 Correlation of semen parameter in subjects with low and high ORP.

	Donors (n = 84)			Patients (n = 301)		
Semen Parameters and reference values	*ORP ≤5.14	ORP>5.14	P value	ORP ≤5.14	ORP>5.14	P value
Sperm concentration (>15 X 106/mL)	52.85 ± 5.14	47.30 ± 13.64	0.84	52.09 ± 3.31	3.70 ± 0.43	<0.001
Total sperm count (>22 X106)	131.55 ± 15.12	94.22 ± 32.57	0.54	158.64 ± 10.75	11.99 ± 1.41	<0.001
Total motility (>32%)	56.6 ± 1.5	62.5 ±6.1	0.33	46.2 ± 1.2	26.17 ± 1.84	<0.001
Normal morphology (>4%)	6.9 ± 0.7	8.3 ± 1.8	0.41	5.2 ± 0.4	3.3 ± 0.3	0.001

Values are mean ± SE; P< 0.05 considered significant by Wilcoxon's rank sum test. *ORP = mVolts/10^6 sperm/ml.

Example 9. Varicocele-induced male infertility-a mitochondrial disease.

OBJECTIVE: Impaired respiratory chain, oxidative phosphorylation, mitochondrial protein import, alterations of the inner mitochondrial membrane composition and defects of mitochondrial dynamics are characteristics of mitochondrial disease. Proteomic data on comparative proteomic analysis of sperm proteins identified 22 differentially expressed proteins (DEP) of mitochondrial origin. Proteins involved in mitochondrial organization (LETM1, EFHC1 and MIC60), import receptor TOM22, 3 crucial subunits of electron transport chain (ETC) and the core enzymes of carbohydrate and lipid metabolism were under-expressed in the varicocele group. In varicocele, stagnation in the testicular microcirculation inducing hypoxic-ischemic degenerative changes in all cell types in the sperm production site. During hypoxia, superoxide production at low oxygen concentrations results in oxidative stress (OS). With this background, hypoxia-mediated

OS is involved in sperm dysfunction in varicocele due to impaired blood supply to the testis.

DESIGN: Validation of key DEP by Western blot (WB) analysis fertile controls (n=10) using the MiOXSYS analyzer. Expression profile of ETC complexes, cAMP-dependent protein kinase A regulatory subunit a (PKARIA) and DEPs responsible for sperm function were validated by WB and indirect immunofluorescence (IF) (n=5 each; for patients and controls). Relative intensity of each band was calculated using I-image software.

RESULTS: All proteins studied were under expressed in varicocele group together with an increase in ORP. These are shown in Table 2. WB data was supported by IF findings.

CONCLUSIONS: Impaired mitochondrial function in infertile men with varicocele leads to OS and sperm dysfunction. Reduction in cAMP level due to declining ATP synthesis results in deregulated protein kinase initiating a vicious cycle by impairing mitochondrial activity. Therefore, along with conventional management, therapy targeted towards mitochondria will improve the treatment outcome in infertile men with varicocele.

Table 2. Protein expression in spermatozoa of men with varicocele in comparison with fertile donors.

Protein	Functions	Fold decrease
PKAR1A	Cell Signaling	2.01 ($p<0.01$)
NDFSU1	Electron transport chain; ATP Synthesis	2.59 ($p<0.01$)
UQCRC2	Electron transport chain; ATP Synthesis	3.64 ($p<0.05$)
COX-II	Electron transport chain; ATP Synthesis	2.49 ($p<0.4$)
HSPA2	Sperm-zona fusion	1.74 ($p<0.03$)
APOPA1	Activates sperm motility and involved in cholesterol efflux	2.37 ($p<0.009$)
SPA17	Sperm-zona binding	2.16 ($p<0.01$)
ATPase1A4	Involved in membrane transport and sperm motility	2.41 ($p<0.03$)

<u>Example 10</u>

This example demonstrates that varicocelectomy improves oxidation reduction potential (ORP) in infertile men.

Patients with varicocele tend to have poor sperm quality and are at higher risk of being infertile. Although the pathophysiology of infertility in males with varicocele has been extensively studied, the underlying mechanism remains unclear. Oxidative stress has been proposed as one of the pathophysiologic mechanisms. sORP is a recent measure of oxidative stress that has been validated as a diagnostic marker for men with infertility.

A prospective, case control study of 43 infertile patients with clinically grade 2-3 varicocele attending a male infertility clinic was conducted. All patients underwent microsurgical subinguinal varicocelectomy. Full medical history and clinical examination were obtained from patients. Semen samples were done using WHO 5th edition guidelines and ORP levels were measured by a MiOXSYS^{TM} analyzer before the surgery and 3 months after. The results were compared by Wilcoxon rank sum test and Spearman's correlation test and a P value < 0.05 was considered significant.

Table 3 compares results of semen parameters and sORP values before and 3 months after the surgery (n=43). All semen parameters (concentration, total motility and normal form of sperm) were found to improve after the surgery but not statistically significantly, except for the sORP that was significantly improved after the surgery (10.4 ± 3.3 vs 4.6 ± 1.1, p value <0.001). Table 4 shows the correlation between semen parameters and sORP before and after surgery. All semen parameters (concentration, total motility and normal form) were significantly negatively correlated with sORP pre-operatively. However, only sperm count maintained this significant negative correlation post operatively.

Table 3: Comparison between semen parameters and sORP values before and after surgery (n=43)

	Before Surgery	**After Surgery**	**P value**
Sperm Count	25.2 +/- 3.4	29.9 +/- 3.1	0.029
Total Motility	44.8 +/- 3.1	46.1 +/- 2.3	0.645
Progressive Motility	9.3 +/- 1.6	10.3 +/- 1.6	0.64
Normal forms	5.5 +/- 1.6	3.6 +/- .04	0.893
sORP	10.4 +/- 3.3	4.6 +/- 1.1	<0.001

Table 4: Correlation between semen parameters and sORP before and after surgery

	Before Surgery	**After Surgery**
Sperm Count	-0.871**	-0.827**
Total Motility	-0.359**	-0.288
Progressive Motility	-0.461**	-0.155
Normal forms	-0.526**	-0.332*

Spearman's correlation

* $p<0.05$ ** $p<0.01$

This example demonstrates that varicocele is associated with elevated oxidative stress that is reflected on all semen parameters. The first parameters to be improved after varicocele surgery are sORP and count. ORP as a measure of oxidative stress in infertile patients with varicocele can be used as a prognostic factor for counselling patients before varicocelectomy.

<u>Example 11</u>

This example identifies a dividing point of sORP values of semen samples between men having normal concentration of sperm in a semen sample and men who are considered to be oligospermic (having an abnormally low sperm count).

The sORP value as measured by a MiOXSYS™ device of 153 men having a normal sperm count was measured and values expressed as mV/10^6 sperm/mL. The sORP value as measured by a MiOXSYS™ device of 92 men considered to be oligospermic was measured and values expressed as mV/10^6 sperm/mL. Of the 92 oligospermic men, 56 had been diagnosed with varicocele and of those 56, 28 had severe oligospermia. A comparison of the analysis between the two groups is shown in Figure 6. Based on Figure 6, an sORP measurement of 2.67 mV/10^6 sperm/mL or greater is considered to be oligospermic, as well as indicative of abnormalities in other semen-related parameters.

All of the documents cited herein are incorporated herein by reference.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following exemplary claims.

What is claimed is:

1. A method of treating infertility in a male subject with varicocele, comprising:
   a. measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject;
   b. comparing the measured sORP to a reference value; and
   c. if the measured sORP value is an elevated sORP as determined by the comparison, administering therapy to improve mitochondrial function.
2. The method of claim 1, wherein the therapy to improve mitochondrial function comprises administering an antioxidant to the subject.
3. The method of claim 2, wherein the therapy to improve mitochondrial function comprises administering an antioxidant selected from the group consisting of L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, vitamin B12, zinc, alpha lipoic acid, B-vitamins; fish or krill oil, glutathione, magnesium, nicotinamide, riboside; pyrroloquinoline quinone and combinations thereof to the subject.
4. The method of claim 1, wherein the elevated sORP is an sORP of 2.67 $mV/10^6$ sperm/mL or greater.
5. The method of claim 1, wherein the subject has clinical grade 2 or 3 varicocele.
6. The method of claim 1, wherein the subject has clinical grade 3 varicocele.
7. The method of claim 1, wherein the step of measuring the sORP comprises liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.
8. A method of determining treatment for a male subject having infertility, comprising:
   a. evaluating the subject to determine the presence of a varicocele in the subject;
   b. measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject;
   c. comparing the measured sORP to a reference value; and
   d. if the measured sORP value is an elevated sORP as determined by the comparison, performing a varicocelectomy on the subject to improve fertility of the subject.
9. The method of claim 8, wherein the subject has clinical grade 2 or 3 varicocele.

10. The method of claim 8, wherein the subject has clinical grade 3 varicocele.

11. The method of claim 8, wherein the step of measuring the sORP comprises liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.

12. The method of claim 8, wherein the elevated sORP is an sORP of about 2.67 $mV/10^6$ sperm/mL or greater.

13. A method of determining the effectiveness of a varicocelectomy procedure on an infertile male subject at improving fertility, comprising:

a. measuring the static oxidation-reduction potential (sORP) of a semen sample from the subject prior to the procedure;

b. measuring the sORP of a semen sample from the subject after the procedure;

c. wherein if the sORP of the semen sample after the procedure is less than the sORP of the semen sample before the procedure, the procedure was effective at improving the fertility of the subject.

14. The method of claim 13, wherein the subject has clinical grade 2 or 3 varicocele prior to the procedure.

15. The method of claim 13, wherein the subject has clinical grade 3 varicocele prior to the procedure.

16. The method of claim 13, wherein the step of measuring the sORP comprises liquefying the semen sample and measuring the sORP of the liquified semen sample as an sORP/sperm cell concentration value within 120 minutes of liquefying the semen sample.

17. The method of claim 13, wherein prior to the procedure, the sORP is an sORP of about 2.67 $mV/10^6$ sperm/mL or greater, and after the procedure, the sORP is an sORP of about 2.67 $mV/10^6$ sperm/mL or less.

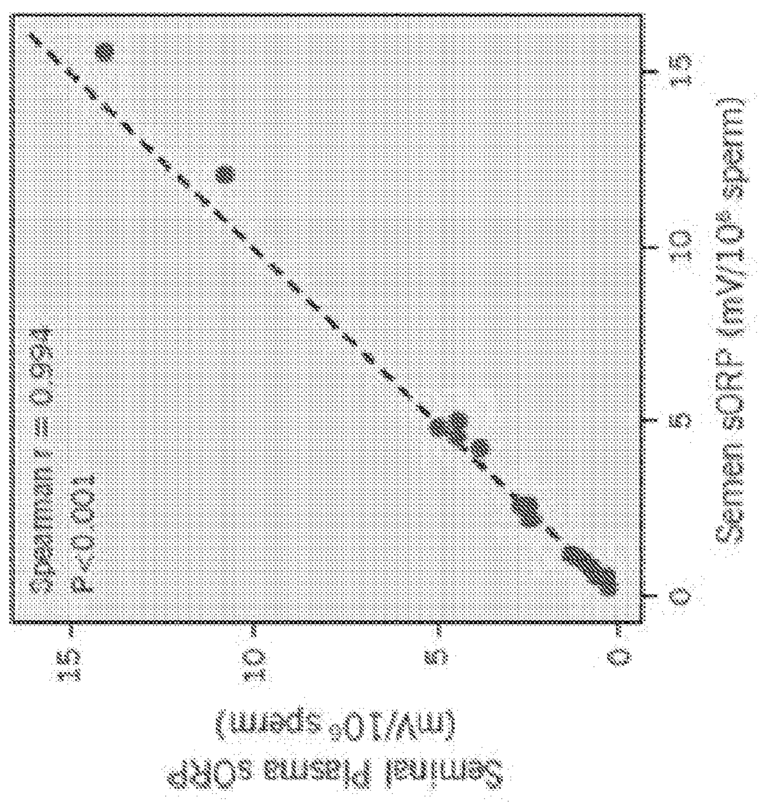
Spearman r = 0.994
P<0.001
Semen sORP (mV/10⁶ sperm)
Seminal Plasma sORP
(mV/10⁶ sperm)
0
5
10
15

Figure 1

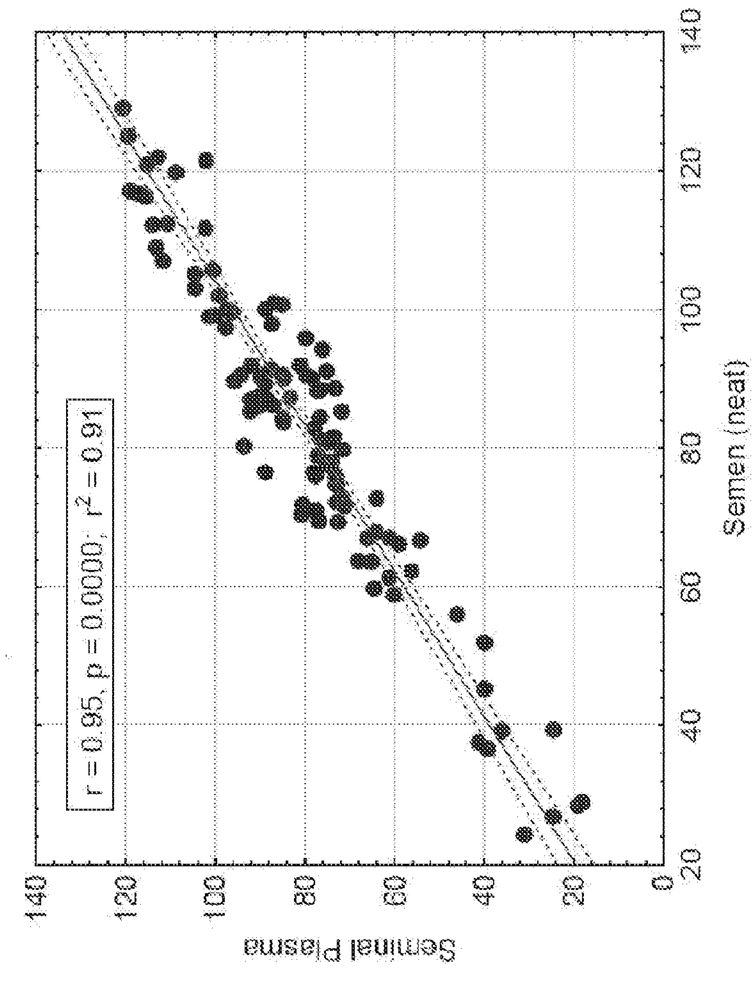
r = 0.95, p = 0.0000; r^2 = 0.91
Seminal Plasma
Semen (neat)
0
20
40
60
80
100
120
140

Figure 2

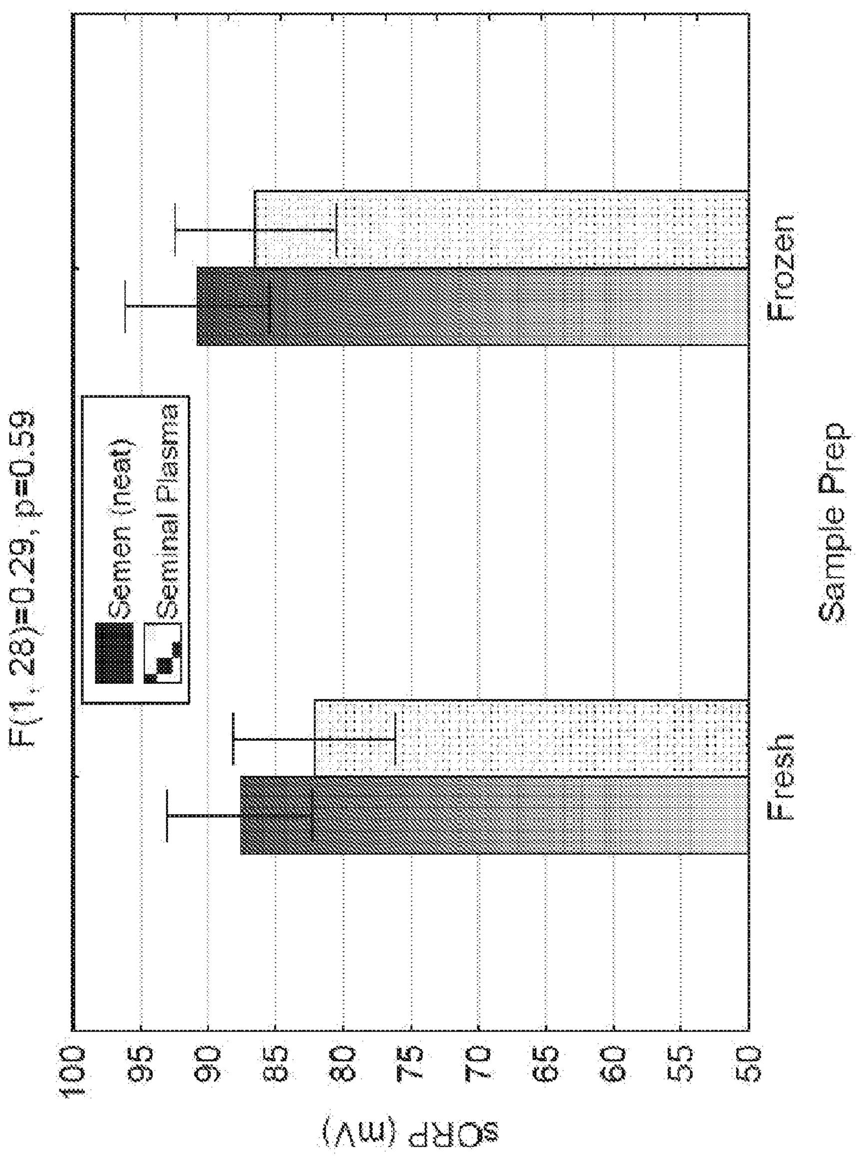
F(1, 28)=0.29, p=0.59
Semen (neat)
Seminal Plasma
sORP (mV)
100
95
90
85
80
75
70
65
60
55
50
Fresh
Frozen
Sample Prep

Figure 3

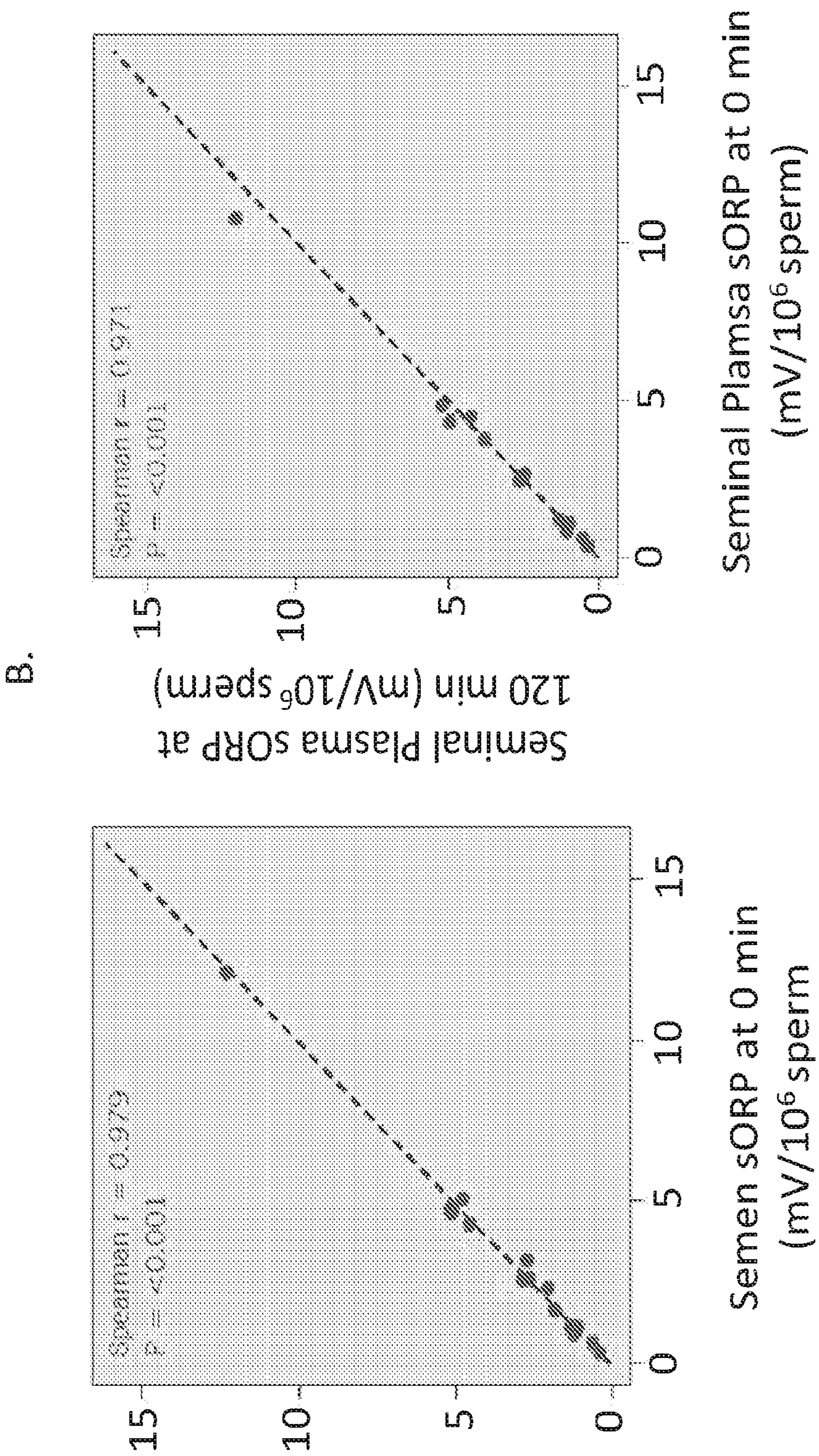
A.
Semen sORP at 120 min (mV/10^6 sperm)
Semen sORP at 0 min (mV/10^6 sperm
Spearman r = 0.979
P = <0.001
0
5
10
15
B.
Seminal Plasma sORP at 120 min (mV/10^6 sperm)
Seminal Plamsa sORP at 0 min (mV/10^6 sperm)
Spearman r = 0.971
P = <0.001
0
5
10
15

Figure 4

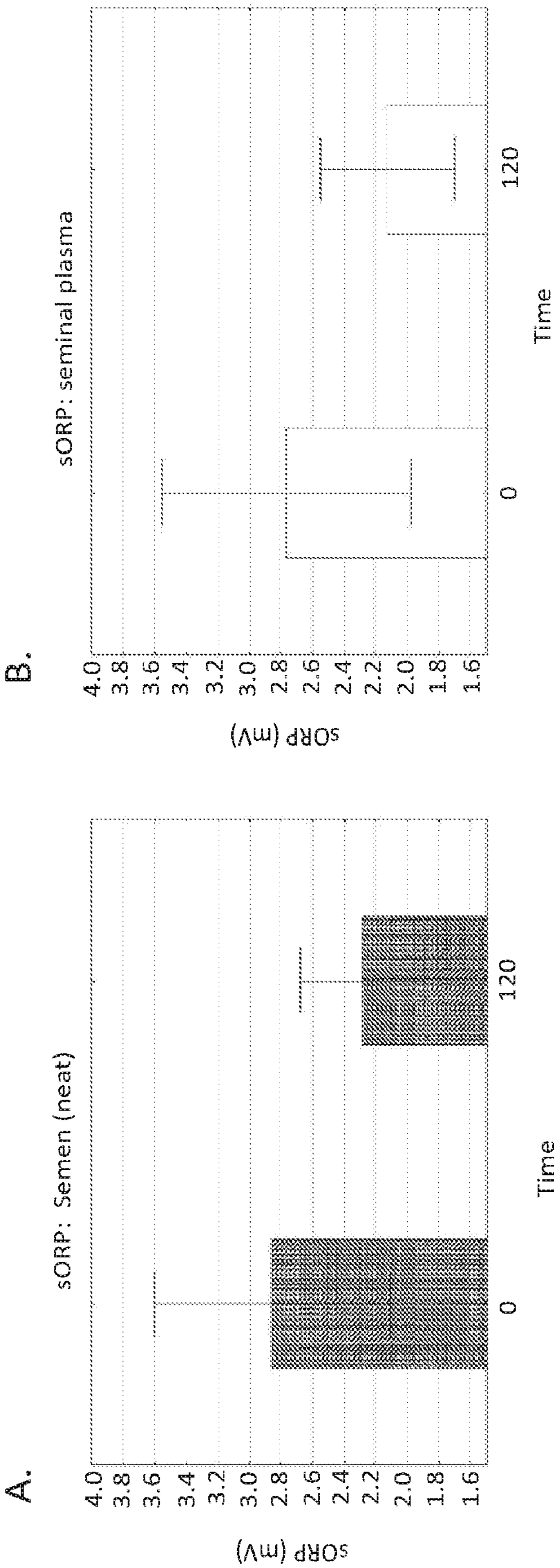
A.
sORP: Semen (neat)
sORP (mV)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
0
120
Time
B.
sORP: seminal plasma
sORP (mV)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
0
120
Time

Figure 5

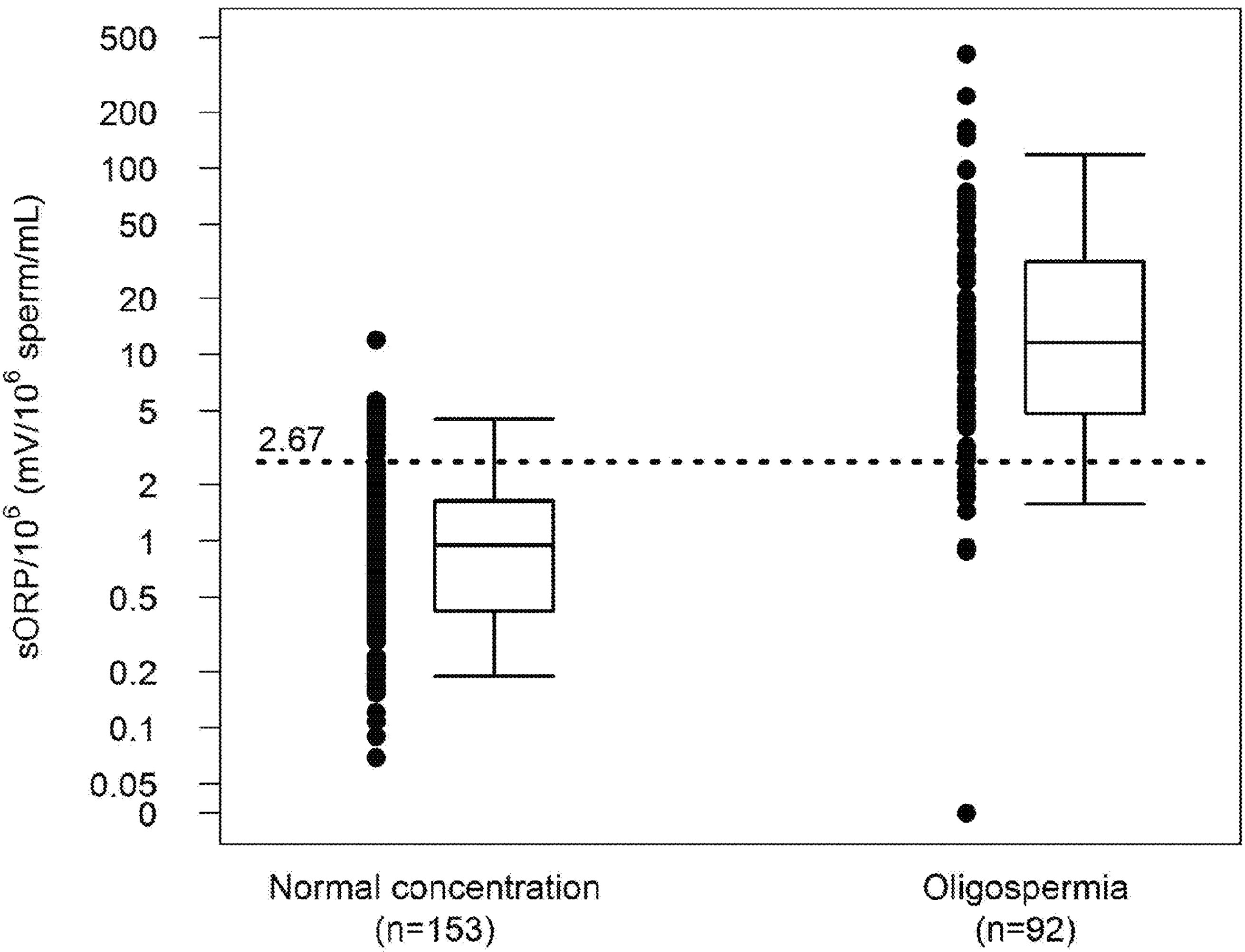
500
200
100
50
20
10
5
2
1
0.5
0.2
0.1
0.05
0
sORP/10^6 (mV/10^6 sperm/mL)
2.67
Normal concentration
(n=153)
Oligospermia
(n=92)

Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/058479

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/416 A61B10/00 G01N33/487
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/085997 A2 (AYTU BIOSCIENCE INC [US]) 2 June 2016 (2016-06-02) page 9, lines 13-26 page 17, line 9 - page 18, line 33 page 19, line 15 - page 20, line 24 page 29, line 27 - page 32, line 19; examples 1-2 ----- -/--	13-17

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2019	04/02/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lazar, Zala

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ASHOK AGARWAL ET AL: "Oxidation-reduction potential of semen: what is its role in the treatment of male infertility?", THERAPEUTIC ADVANCES IN UROLOGY FEB 2013, vol. 8, 28 June 2016 (2016-06-28), pages 302-318, XP055473637, ISSN: 1756-2872, DOI: 10.1177/1756287216652779Therapeutic abstract page 303, column 1, paragraph 2-3 page 305, column 2, paragraph 1 - page 307, column 1, paragraph 1 page 309, column 1, paragraph 2 - page 311, column 1, paragraph 2 -----	13-17

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/058479

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-12
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 1-12

A meaningful search is not possible on the basis of claims 1 to 12 because: (i) claims 1 to 12 are directed to a method for treatment of the human or animal body by therapy (claim 1: administering therapy, administering antioxidant; claim 8: performing varicocelectomy) - Rule 39.1(iv) PCT; and (ii) claims 8 to 12 are directed to a method for treatment of the human or animal body by surgery (claim 8: performing varicocelectomy) - Rule 39.1(iv) PCT.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/058479

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