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Examining the effects of a multicomponent oral antioxidant therapy on semen parameters and seminal oxidative stress in males with idiopathic infertility

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Abstract:

The aim of this study to assess the efficacy of a ten constituent oral antioxidant formula, on various semen parameters and seminal oxidative stress in males with idiopathic infertility. Single center, prospective, comparison the efficacy of a multicomponent oral antioxidant drug and a matching placebo, Thirty infertile males were included and subjected to detailed history taking, complete general and genital examination, conventional semen analysis, seminal oxidative stress assay by the MiOXSYS system and finally receiving an oral antioxidant for one month followed by one month washout period, then a seemingly identical placebo was given.

Keywords: Antioxidant, male infertility, oxidative stress**.**

1. Introduction:

Assessment of male fertility potential via conventional semen parameters seems to be an oversimplification of the man's ability to father a child due to large inter- and intra-individual variations in traditional measures [1], [2]**.** Oxidative stress (OS), characterized by an overabundance of reactive oxygen species (ROS) or a deficiency of antioxidants, is one of the major causes of male infertility [3],[4]. Current assays for seminal OS include ROS assay, total antioxidant capacity (TAC) assay or malondialdehyde (MDA) assay, all of which have their own drawbacks [5].

Treatments of oxidative stress, including oral antioxidants and varicocelectomy, have been studied widely in patients with

varicocele associated male sub fertility and unexplained male infertility. Ideally, an oral antioxidant should reach high concentrations in the reproductive tract and restore vital elements important for spermatogenesis. Additionally, the antioxidant supplement should augment the scavenging capacity of seminal plasma and reduce levels of seminal ROS [6],[7].

In a randomized, triple-blind, placebo-controlled clinical trial, Haghighian and colleagues [8] observed that alpha lipoic acid supplementation in infertile men improved semen parameters and seminal levels of TAC and MDA.

Additionally, in infertile men, oral antioxidant therapy with Carni-Q-Nol (L-carnitinefumarate, ubiquinol, vitamin E, and vitamin C) twice or thrice daily decreased sperm pathology after three months, improved sperm density, increased (ubiquinone + ubiquinol) and α-tocopherol, and decreased OS levels [9].

Measurement of oxidation-reduction potential (ORP) by the MiOXSYS™ system (AytuBioScience, Inc.) presents a novel and comprehensive measure of seminal oxidative stress. It has a number of advantages over existing semen quality measures. The MiOXSYS system and measurement of ORP represent an invaluable clinical tool that obviates the need for complicated oxidative stress assays. The system facilitates wider application of oxidative stress assays in both clinical and research settings. The incorporation of ORP measurement into the armamentarium of the male infertility specialist will facilitate management of infertile couples by identifying candidates who may benefit from treatment of oxidative stress [10].

2. Patients and Methods:

The present study includes thirty treatment couples with failed conception for more than one year despite regular unprotected coitus.

2.1 Inclusion criteria:

1. Primary infertility more than one year.

2. Age between 20 and 50 years

3. High normalized ROS level (1.894)

4. Main semen abnormality being asthenozoospermia (< 40% total motility and < 32% progressive motility), either isolated or with oligozoospermia (sperm concentration less than 15 million sperm /ml).

5. Normal genital examination with no evidence of varicocele, cryptorchidism.

6. Normal serum follicle-stimulating hormone (FSH), total and free testosterone level, LH and prolactin level.

7. No history of smoking, drug /alcohol abuse, or occupational exposure to possible reproductive toxins.

8. Intact sexual function and ability to provide semen samples.

Exclusion criteria

1. Absolute asthenozoospermia (total sperm immotility).

2. Evidence of genitourinary tract infection (Leucocytospermia, pyuria, etc…) or antisperm antibodies.

3. Morbid obesity or spinal cord injury.

4. Hypogonadism or genetic disorders.

5. Severe uncontrolled medical diseases (e.g. diabetes, hypertension, liver, or kidney diseases).

6. Use of cytotoxic drugs, or immunosuppressants.

7. Known hypersensitivity to any of the drug components.

2.2 All patients were subjected to: After signing an informed consent, each infertile male was managed as follows:

(1) Full history taking.

(2) Complete general and genital examination.

(3)Semen analysis: Prior to treatment, each patient was evaluated by at least two semen samples collected and analyzed according to the World Health Organization [11] standards.

(4) Seminal oxidative stress (redox imbalance) assay:

Prior to treatment, ORP was measured using galvanostat-based technology—the MiOXSYS System (AytuBioScience, Inc.; Supplemental).

ORP measures the transfer of electrons from a reductant (or antioxidant) to anoxidant. Briefly, 30 μL of liquefied semen at room temperature were applied to the MiOXSYS sensor. The sensor was preinserted into the MiOXSYS analyzer; to begin automatic measurements. The MiOXSYS System provides two measures of oxidative stress. Static ORP (sORP), measured in millivolts, is the integrated measure of the existing balance between total oxidants and reductants in a biological system. After this initial sORP reading is recorded, the analyzer automatically applies a small current sweep to the sample, resulting in the exhaustion of all antioxidant species, providing a measure of antioxidant capacity reserve (cORP), measured in microcoulombs (mC). Data then was normalized to sperm concentration to control for differences in cell numbers. Thus, data was presented as millivolts/106 sperm/milliliter for sORP and microcoulombs/106 sperm/ milliliter for cORP (Agarwal et al., 2016).

(5) Hormonal profile Serum FSH, luteinizing hormone (LH), prolactin, total and free testosterone, estradiol levels, tzi ,sdi ,acrosomal index were assessed.

(6) Receiving either an antioxidant drug or a placebo:

Oxifree oral therapy (Devartlab pharmaceutical company, Cairo, Egypt) for 1 month followed by 1 month washout period then a seemingly identical placebo for 1 month.

(7) Recording cases with positive pregnancy test:

Measuring the rates of positive pregnancy test by natural conception after admistration of antioxidant supplements.

Oxifree oral therapy: Oxifree pills - containing a fixed dose combination of 55 mcg selenium+ 11mg zinc+ 45 mcg molybdenum+1.8 mg β- carotene+ 90 mg vitamin C+ 15 mg vitamin E+100 mg mixed bioflavonoids+ 40 mg proanthocyanidins+ 450 mg omega 3+ 40 mg alpha lipoic acid- had been given as once-daily, single-tablet therapy 1 hour before breakfast.

A matching placebo, manufactured in tablet form by the company, was received once daily before breakfast. Antioxidant drug and placebo were identical in appearance and were given to patients in numbered bottles.

For each patient, successive monitoring of basic semen parameters (spermconcentration, total and progressive motility, etc…) and seminal OS (via theMiOXSYS™ system; AytuBioScience, Inc.) was conducted at baseline, after end of treatment and after end of placebo.

Statistical methodology:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test. For comparison of serial measurements within each patient the non-parametric Wilcoxon signed rank test was used [12]. For comparing categorical data, Chi square ($X2$) test was performed. Exact test was used instead when the expected frequency is less than five [13]. Correlations between quantitative variables were done using Spearman correlation coefficient [14].P-values less than 0.05 were considered as statistically significant.

3. Results:

Table (1): Comparison of semen analysis between baseline and 2nd semen analysis of the studied population; (N= 30):

SEMEN ANALYSIS	Mean ±SD		P-value
	Baseline analysis	2nd analysis	
Sperm Concentration	7.28 ±6.60	12.28 ±11.18	0.001***
Sperm Total Count (Million)	23.48 ±25.45	36.99 ±38.17	0.009***
Total Motility (%)	18.27 ±12.61	26.20 ±14.42	0.001***
ROS (Mv)	10.17 ±8.24	4.57 ±5.31	<0.001***

Mean sperm total count significant p-value (0.009). Total sperm significant p-value (0.001). ROS significant p-value (<0.001). Sperm Concentration significant p-value (0.001).

Table (2): Comparison of semen analysis between baseline and 3rd semen analysis of the studied population; (N= 30):

SEMEN ANALYSIS	Mean ±SD		P-value
	Baseline	3rd visit	
Sperm Concentration	7.28 ±6.60	15.03 ±15.05	0.001***
Sperm Total Count (Million)	23.48 ±25.45	45.42 ±45.61	0.004***
Total Motility (%)	18.27 ±12.61	28.33 ±17.34	< 0.001***
Normal Morphology (%)	2.73 ±2.60	4.47 ±3.39	0.013***
ROS (Mv)	10.17 ±8.24	2.61 ±3.93	< 0.001***
Vitality(%)	50.56 ±19.82	58.62 ±11.74	0.03***

Mean sperm total count significant p-value (0.004). Total sperm motility significant p-value (<0.001). Normal morphology significant p-value (0.013). ROS significant p-value (<0.001). Sperm Concentration significant p-value (0.001). Vitality significant p-value (0.03).

Table (3): Comparison of semen analysis between second and third semen analysis of the studied population; (N= 30):

SEMEN ANALYSIS	Mean ±SD		P-value
	2nd visit	3rd visit	
Normal Morphology (%)	2.87 ±3.22	4.47 ±3.39	0.049***
ROS (Mv)	4.57 ±5.31	2.61 ±3.93	<0.001***

Normal morphology significant p-value (0.049) . ROS significant p-value (<0.001).

Table (4): Correlation between patients' age and semen parameters in baseline semen analysis; (N= 30):

SEMEN ANALYSIS	patients' age	
	Correlation Coefficient	p-value
Vitality	-0.518	0.006***

Patients' age (r= -0.518, p= 0.006).

Table (5): Correlation between ROS level (basal, after treatment, after placebo) and other semen parameters in baseline semen analysis; (N= 30):

		ROS (mV) Baseline analysis	ROS (mV) (2nd semen analysis)	Normalized ROS (for sperm conc.) (2nd semen analysis)	ROS (mV) (3rd semen analysis)
Sperm Total Count (million)	**R**	-0.687	-0.505-	0.075	-0.225-
	P value	< 0.001***	0.004***	0.693	0.231
Progressive motility (%)	**R**	0.224	0.261	-0.719-	0.298
	P value	0.235	0.163	< 0.001***	0.109
Sperm Concentration	**R**	-0.758	-0.591-	0.075	-0.371-
	P value	< 0.001***	0.001***	0.692	0.044***

ROS (mV) Baseline analysis was negatively correlated with sperm Total Count (million) where (r= -687, p=0.001) and negatively strong correlated with Sperm concentration where (r= -758, p=0.001).

ROS (mV) (2nd semen analysis) was negatively moderate correlated with sperm total count (million) where (r= -505, p=0.004) and negatively correlated with sperm concentration where (r= -591, p=0.001). Normalized ROS (for sperm conc.) in (2nd semen analysis) was negatively strong correlated with sperm progressive motility (%) where (r= -719, p=0.001).

ROS (mV) in (3rd semen analysis) was negatively moderate correlated with sperm concentration where (r= -371, p=0.044).

Table (6): Correlation between ROS level (basal, after treatment, after placebo) and other semen parameters in 2nd visit semen analysis; (N= 30):

		ROS (mV) Baseline analysis	ROS (mV) (2nd semen analysis)	Normalized ROS (for sperm conc.) (2nd semen analysis)	ROS (mV) (3rd semen analysis)
Volume	**R**	0.379	0.283	-0.054	0.408
	P value	0.039***	0.130	0.777	0.025***
Sperm Total Count (million)	**R**	-0.464	-0.688	0.182	-0.445
	P value	0.010***	< 0.001***	0.335	0.014***
Normal morphology (%)	**R**	-0.259	-0.332	0.234	-0.581
	P value	0.167	0.073	0.213	0.001***
	P value	0.191	0.200	0.626	0.387
Sperm Concentration	**R**	-0.584	-0.727	0.204	-0.574
	P value	0.001***	< 0.001***	0.279	0.001***

ROS (mV) in Baseline analysis was positively correlated with semen volume where (r= 0.379, p= 0.039), negatively correlated with Sperm Total Count (million) where (r= -0.464, p= 0.010) and negatively moderate correlated with Sperm Concentration where (r= -0.584, p= 0.001).

ROS (mV) in 2nd semen analysis was negatively correlated with Sperm Total Count (million) where (r= -0.688, p= 0.001) and negatively strong correlated with Sperm Concentration where (r= -0.727, p= 0.001).

ROS (mV) in (3rd semen analysis) was positively correlated with semen volume where (r= 0.408, p= 0.025), negatively correlated with Sperm Total Count (million) where (r= -0.445, p= 0.014) and negatively correlated with Sperm Concentration where (r= -0.574, p= 0.001).

Table (7): Correlation between ROS level (basal, after treatment, after placebo) and other semen parameters in 3rd visit semen analysis; (N= 30):

		ROS (mV) Baseline analysis	ROS (mV) (3rdsemen analysis)	Normalized ROS (for sperm conc.) (3rdsemen analysis)	ROS (mV) (3rd semen analysis)
Sperm Total Count (million)	**R**	-0.586-	-0.460-	0.215	-0.399-
	P value	0.001***	0.011***	0.255	0.029***
SDI	**R**	0.174	0.105	-0.446-	0.074
	P value	0.427	0.632	0.033***	0.736
Sperm Concentration	**R**	-0.655-	-0.560-	0.279	-0.521-
	P value	0.001***	0.001***	0.135	0.003***

ROS (mV) in Baseline analysis was negatively correlated with Sperm Total Count (million) where (r= -0.586, p= 0.001) and negatively correlated with Sperm Concentration where (r= -0.655, p= 0.001).

ROS (mV) in 2nd semen analysis was negatively correlated with Sperm Total Count (million) where (r= -0.460, p= 0.011) and negatively correlated with Sperm Concentration where (r= -0.560, p= 0.001).

Normalized ROS (for sperm conc.) in (2nd semen analysis) was not correlated with SDI where (r= -0.446, p= 0.033).

ROS (mV) in 3rd semen analysis was negatively correlated with Sperm Total Count (million) where (r= -0.399, p= 0.09) and negatively correlated with Sperm Concentration where (r= -0.521, p= 0.003).

4. Discussion:

The rationale for oral antioxidant therapy is because seminal OS is due to increased ROS production and/or decreased levels of seminal antioxidants [15].The different oral antioxidants available belong to the exogenous antioxidant category and they include Vitamin C, Vitamin E, coenzyme Q10, N-acetyl cysteine, carnitines, trace elements such as zinc, selenium, pentoxifylline, and a combination of these oral antioxidants [16]**.**The role of oxidative stress and especially the therapeutic use of antioxidative vitamins have been widely considered for fertility disturbances [17]**.** Also, deleterious effects of reactive oxygen species and other oxidant molecules on sperm motility and membrane integrity have been well documented [18].

Numerous studies have been conducted to assess the effectiveness of oral antioxidant supplementation for the treatment of male infertility. Most of the studies showed an improvement in one or more of seminal fluid parameters, whereas some studies reported no positive effect [19]**.**

This study was performed to assess the efficacy of a ten constituent oral antioxidant formula (oxifree pills), on various semen parameters and seminal oxidative stress (redox imbalance) detected by galvanostat based technology -the MiOXSYS system in males with idiopathic asthenozoospermia and oligoasthenozoospermia.

According to findings; mean sperm total count was significantly increased after oxifree antioxidant treatment as compared with the baseline assessment and further increase was detected in the 3rd analysis but without a statistically significant difference. This result was accordance with a few studies in the same context, however; many other studies did not report any significant increase in the mean sperm total count [20]**.**

Total sperm motility was significantly increased after the treatment period with oxifree antioxidant. This result is consistent with previous studies reporting improvement of sperm motility following oral antioxidant supplementation for the treatment of male infertility. Wirleitner and his colleagues [21] conducted a study aimed to investigate the influence of an oral anti-oxidative supplementation on sperm quality; and they reported significant improvement in sperm motility at two - twelve months after an antioxidative

supplementation. Also many other researchers in the same context reported significant improvement in sperm motility after different oral antioxidative supplementation [22][23][24]**.**

While on the opposite side; Bozhedomovand his colleagues [25]reported no effect on the sperm motility after three-four months of treatment**.** Improvement in sperm motility has been shown mostly in researches considering mixture of more antioxidants such as selenium and vitamin E [26]**.**

Reactive oxygen species (ROS) play an important role in male infertility and are proved to be higher in infertile men; antioxidants could oppose their effect [27].In the present study; ROS was significantly decreased in the second semen analysis after treatment as compared with the baseline assessment and further significant decrease in ROS was detected in the 3rd analysis after placebo; ROS was negatively correlated with sperm total Count (million) and negatively correlated with sperm concentration. [28] reported the same finding.

Treatment significantly reduced ROS in a study conducted to detect the effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in sub-fertile men [29].

In another study; level of ROS decreased after antioxidant mixtures [30].

Low sperm concentration or oligozoospermia is defined as concentration less than 15x106 spermatozoa/ml according to WHO reference value from 2010 [31]**.**

Sperm Concentration in the present study was significantly increased after treatment as compared with the baseline assessment; vitality was negative correlated with patients' age. Many researches showed significant improvements in sperm concentration after oral intake of different antioxidants [32][33][34]. Most of these researches investigated combination of different antioxidants, like L-carnitine, coenzyme Q10 (CoQ10), vitamin C, vitamin E, zinc (Zn), selenium (Se), and so forth.

Regarding sperm morphology; WHO reference values from 1999 defined teratozoospermia as less than 14% of normal shape and form spermatozoa according to strict Kruger' criteria [35].

Although WHO reference values from 2010 define teratozoospermia as reduction of percentage of sperm with normal morphology (4%) assessed by light microscopy; strict Kruger criteria are still used as reference value for assessing sperm morphology [36]**.** In the

present study; sperm morphology was improved between the baseline and final assessment of patients with a statistically significant p-value (0.013). This was supported by many studies***;*** [37][38]. On the other hand; in a similar study to assess the efficacy and safety of a complex of nutritional supplements with antioxidant activity (L-carnitine, acetyl-L-carnitine,fructose, citric acid, selenium, coenzyme Q10, zinc, ascorbic acid, cyanocobalamin, folic acid) in primary infertile patients with idiopathic astenoteratozoospermia; they reported non-significant improvement in sperm morphology [39]**,** same findings were reported by[40]who observed no detected effect on sperm morphology.

5. Conclusion and Recommendations:

Based on findings, it can be established that the use of oral antioxidant formula (oxifree pills) can be an efficacious strategy to handle male infertility; we can say that this formulation can improve the parameters of sperm quality. Antioxidants play an important role in protecting semen from ROS and can improve basic sperm parameters in case of idiopathic oligo-astheno-terato-zoospermia

The study recommends future directions include identifying the underlying molecular mechanisms that explain the specific effects of some antioxidants on semen parameters, optimizing the dose and duration of therapy, know how long the effect of treatment will last before repeating it and the choice between individual or combined therapy, and measuring ROS and oxidants/antioxidants in seminal plasma, investigating further the usefulness for therapeutic guidance.

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