

Fixed Dose Combination Therapy of Antioxidants in Treatment of Idiopathic Oligoasthenozoospermia: Results of a Randomized, Double-blind, Placebo-controlled Clinical Trial

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ABSTRACT

Introduction: To assess the effectiveness and safety of fixed dose combination (FDC) of antioxidants in treatment of idiopathic oligoasthenozoospermia.

Materials and methods: Placebo-controlled, Double-blind, randomized, Parallel three arm, Multicentric trial.

Setting: Fertility clinics of five centers across India.

Patients: One hundred and thirty-eight male subjects, aged between 21 and 50 years and subfertile for 1 year or more with the following baseline sperm selection criteria: Concentration <15 million/ml and total sperm motility <40%.

Intervention(s): Eligible subjects were randomized to either of the three arms in a double-blind manner, i.e. arm 1 was given 2 tablets twice daily of FDC of antioxidants (coenzyme-Q10: 50 mg, L-carnitine: 500 mg, lycopene: 2.5 mg and zinc: 12.5 mg); arm 2 was given 1 tablet of FDC of antioxidants and one tablet of placebo twice daily and arm 3 was two tablets twice daily of matching placebo all for 180 days.

Main outcome measure(s): The primary outcome measures were improvement in sperm count and sperm motility, whereas pregnancy rate was the secondary efficacy outcome.

Results: Compared to placebo, a statistically significant improvement was seen in sperm count (14.8-26.35 in arm 1 and 14.37-24.8 million/ml in arm 2, $p < 0.0001$), and sperm total motility (39.2-51.6% in arm 1 and 38.4-50.1% in arm 2, $p < 0.0001$), at 90 days, and treatment further improved these parameters at day 180. No intergroup difference was seen between arm 1 and arm 2. Mild adverse event of upper gastrointestinal discomfort by 8 subjects (three in arm 1; one in arm 2 and four subjects in arm 3) were reported. No serious adverse event was seen in the study.

Conclusion: Exogenous administration of fixed dose combination of antioxidants is a safe and effective therapy in improving the male subfertility.

Keywords: Oligospermia, Asthenozoospermia, Ubiquinone (coenzyme Q10), L-carnitine, Zinc, Lycopene.

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INTRODUCTION

Worldwide, more than 70 million couples suffer from infertility and the majority of these reside in developing countries. Male subfertility may be contributing to total infertility in large.¹⁻⁴ Male subfertility can be the result of congenital urogenital anomaly, infections of the testes or tract, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors;⁵ however idiopathic male subfertility is found in 30 to 75% of cases.⁶ Semen analysis of subfertility cases often reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). These abnormalities usually occur together and are described as the oligoasthenozoospermia syndrome.

A growing body of evidence shows deleterious role of reactive oxygen species and other oxidative radicals on human sperm.^{7,8} Oxidative injury is observed to be possibly causing the damage of sperm membrane and fragmentation of DNA either at nuclear or mitochondrial levels.

Micronutrients, such as coenzyme Q10 (CoQ10) and L-carnitine have been found as components of the mitochondrial respiratory chain that plays a crucial role both in energy metabolism and as liposoluble chain-breaking antioxidants for cell membranes and lipoproteins.^{9,10} CoQ10 biosynthesis is markedly active in testis,¹¹ and high levels are present in sperm,¹²⁻¹⁴ suggesting a protective role as antioxidant. Some groups have demonstrated reduced levels of CoQ10 in seminal plasma and sperm cells of infertile men with idiopathic and varicocele-associated asthenozoospermia.¹⁵ On this basis, CoQ10 is considered one of the compounds contributing to the total antioxidant buffer capacity of semen, and its decreased levels are deleterious to the system in dealing with oxidative stress.^{16,17}

L-carnitine also has antioxidant characteristics that protect sperm against toxic free radical species. Studies^{18,19} have demonstrated the effectiveness of L-carnitine in

treating male subfertility due to idiopathic or microbial infections by increasing sperm count, motility and semen volume significantly. Carnitine decreases oxidative stress via interaction with arachidonic acid incorporation into phospholipids and protein kinase C-mediated NADPH oxidase system.²⁰ It is also proposed that carnitine exerts antioxidant properties as a result of repairing mechanism by which elevated intracellular toxic acetyl-CoA is removed and fatty acids in membrane phospholipids are replaced.

Lycopene is natural occurring carotenoids that have very potent free radical scavenger properties during oxidative stress,^{21,22} and may play a major role in prevention of oxidative sperm injury.

Zinc had a documented role in testosterone formation, and low zinc intake may be an important etiology for low sperm counts quality of sperm²³ and idiopathic male subfertility.

Though, previously studies have shown the benefits of CoQ10,²⁴⁻²⁷ L-carnitine,²⁸⁻³⁰ lycopene³¹⁻³² and zinc^{33,34} as an individual agent in male subfertility, but so far studies are lacking on its combinational use in male subfertility in Indian population. Therefore, the current study was designed to evaluate the safety and effectiveness of FDC of seminal antioxidants in subjects of idiopathic oligoasthenozoospermia in a randomized double-blind manner.

MATERIALS AND METHODS

The study was approved by Independent Ethics Committee (IEC) for each center and was conducted in accordance with ICH-GCP and declaration of Helsinki rule and was registered in clinical trial registry of India. Before initiation of the study, training was imparted to all the participating centers to keep uniformity in study methods and data collection. All subjects were enrolled in the study after obtaining their written informed consent. Trial were registered to CTRI and registration number were CTRI/2010/091/000574.

Study Population

One hundred and thirty-eight subjects (mean age: 30.74 years, range: 24-45 years) affected by idiopathic oligoasthenozoospermia were enrolled in the study. The subjects were selected at the fertility clinics of five centers across India, i.e. Sooriya Hospital, Saligramam, Chennai; Menz Health Clinic, Noida, UP; Bharti Research Institute of Diabetes and Endocrinology (BRIDE), Karnal, Haryana; Men's Health Clinic, Indore, MP and Southend Fertility and IVF Centre, Holy Angels Hospital, New Delhi, India. All subjects presented with clinical history of primary

infertility for >1 year were underwent medical screening, including history and clinical examination. Each subject was evaluated for testicular volume; varicocele; any anatomic abnormalities of testicular, seminal vesicle and prostatic using ultrasonography; venous spermatic plexus using echocolor Doppler; sperm culture, urethral specimen culture; semen analysis; antispermatozoa antibodies; FSH, LH, prolactin, estradiol and testosterone assay using RIA kits.

A due care was given to rule out any female factor involvement, since all the female partners of participating subjects were undergone ultrasound evaluation for ovary, uterus and fallopian tube anatomy during hysterosalpingography and were free from any abnormalities and ovulated regularly, as formally proved by biphasic basal body temperature and luteal phase progesterone levels. All women denied sexual involvement with another male partner.

Inclusion and Exclusion Criteria

The following criteria were adopted for subjects' eligibility: male subjects with age 21 to 50 years, infertility >1 years, sperm count less than 15 million/ml, sperm total motility <40%,^{35,36} no history of taking therapy for infertility, no history of obstructive azoospermia and regular sexual intercourse with a potentially normal fertile female, willing to sign informed consent and likely to be available for all visits during follow-up period.

Subjects with primary testicular disease, any organic cause for infertility including varicocele, prostatic-vesiculopididymitis, genital infectious disease, planning for any other assisted reproductive techniques (ART) during the study period, serum follicle stimulating hormone FSH >15 mIU/ml, abnormal serum levels of LH, testosterone, estradiol and prolactin, presence of antispermatozoa antibodies, severe oligospermia (<2 million sperm/ml), azoospermia, seminal white blood cells (WBCs) more than 1×10^6 ml, major hepatic and renal disease, myopathy, history of allergy to any ingredient of the formulation, not likely to be available for follow-up, have participated in another clinical trial in the past 3 months and female partners with anatomic or physiological alterations causing subfertility were excluded from the study.

Study Design and Treatment

Subjects matching all the inclusion and exclusion criteria on screening were centrally randomized to one of three treatment arm (arm 1-3), in a 1:1:1 ratio; arm 1 subjects received 2 tablets of FDC antioxidants twice daily, arm 2 subjects received 1 FDC tablet + 1 placebo tablet twice daily and arm 3 subjects received 2 tablets of placebo twice daily.

FDC tablets of antioxidants, (Co-Q10 50 mg, L-carnitine 500 mg, zinc 12.5 mg and lycopene 2.5 mg) and its matching placebo tablets were manufactured by Zyodus Cadila Ltd, Ahmedabad, India. All the subjects were followed up at 3 and 6 months of therapy, and further 6 months follow-up if required. Semen analysis was carried out as recommended by the World Health Organization, 2010.³⁶ Subject compliance and possible side effects were also noted at each visit. The primary efficacy endpoint identified was improvement in sperm count, and sperm motility over baseline compared to placebo. The secondary efficacy endpoint was pregnancy rate.

Semen Analysis

Semen was collected by masturbation after 2 to 3 days of sexual abstinence. Semen analyses were done at baseline, 90 days after enrollment and 180 days after enrollment. A validated and standard laboratory was used in the study for collection and analyses of the semen (manual results were calculated for sperm concentration or count). For motility evaluation percentage of sperm with WHO A, B, C or D motility was assessed by WHO 2010 guidelines criteria.³⁶

Statistical Analysis

Descriptive statistics were computed for demographic and seminal parameters and a statistical comparison was made for seminal parameters. Paired t-test was used to calculate statistical difference between the days 90 and 180 from baseline (day 0). The change from baseline (day 0) in sperms parameter on day 90 and 180 was calculated. Analyses of covariance (ANCOVA) model was used to evaluate the least square mean change from baseline which includes treatment as fixed effect and baseline (day 0) as covariate. Changes were presented using the least-square means derived from the ANCOVA model. A comparison between treatment groups was made using the difference in least-square means and p-values from the ANCOVA model. All the statistical analyses were performed using Statistical Analysis System (SAS) Statistical Package (version 9.1; SAS Institute Inc Cary, North Carolina, 27513-2414 USA).

RESULTS

Of the 200 subjects screened, 138 were enrolled in the study (46 subjects randomized in each group), whereas only 125 (46, 43 and 36 in arms 1, 2 and 3 respectively) completed the study and 13 subjects were lost to follow-up at different stage during the study. The subjects enrolled in this study are for more in number than any of the historic trial with the similar conditions. Data analysis was performed on

125 subjects who completed the entire duration of study. Disposition of the study population is shown in Figures 1 to 4. Demographic characteristics of enrolled subjects are given in details in Table 1. The baseline characteristics of enrolled subjects were statistically comparable in all the three arms: Arm 1 (2 tablets of antioxidant FDC, twice daily), arm 2 (1 tablet of FDC+ 1 tablet of placebo, twice daily) and arm 3 (2 tablets of placebo, twice daily).

Seminal Analysis

Results showed statistically significant improvement in sperm count (increase from 14.8 to 26.35 in arm 1 and 14.37 to 24.8 million/ml in arm 2, $p < 0.0001$) and sperm motility (39.2-51.6% in arm 1 and 38.4-50.1% in arm 2, $p < 0.0001$) in both the treatment arms, at day 90 as compared to their baseline value at day 0, whereas small improvement in seminal parameters was also seen in placebo group (Table 2 and Fig. 2). Treatment for 180 days further improved the seminal parameters over the baseline in both the arms 1 and 2. Furthermore, compared to placebo, improvements in seminal parameters were significantly much more in both the arms of treatment (arm 1 as well as arm 2; Table 3). However, no intergroup difference was seen between the

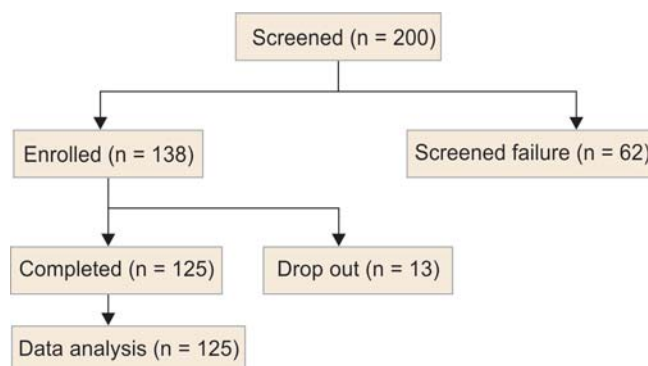


Fig. 1: Disposition of study subjects

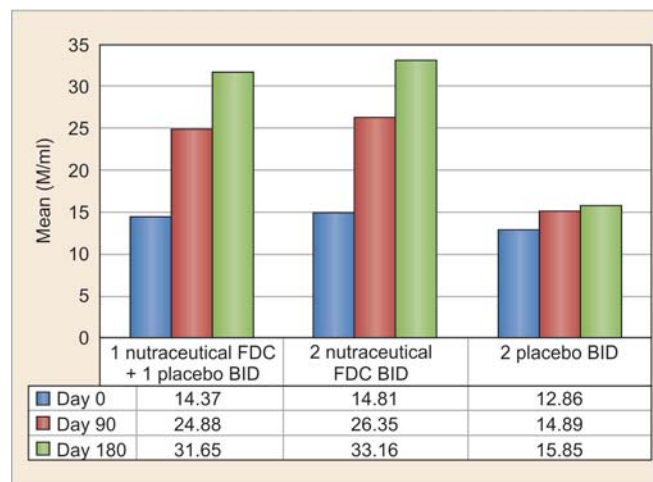


Fig. 2: Sperm count (million/ml) in each arm

two-treatment arms (arm 1 and arm 2) on any of seminal parameters (Table 3).

Spontaneous Pregnancies

A total of 15 spontaneous pregnancies observed during the study (6 pregnancies in arm 1 and 7 pregnancies in arm 2, and 2 pregnancies in arm 3) (Table 2). Four pregnancies in arm 1 occurred after 3 months and 2 after 5 months, whereas

in arm 2, two pregnancies occurred after 2 months, 3 after 3 months and 2 after 4 months. Of the 2 pregnancies of placebo group, one occurred after 4 months and one after 5 months.

Safety Assessments

No serious adverse event was observed in the study. Mild adverse event of upper gastrointestinal discomfort by

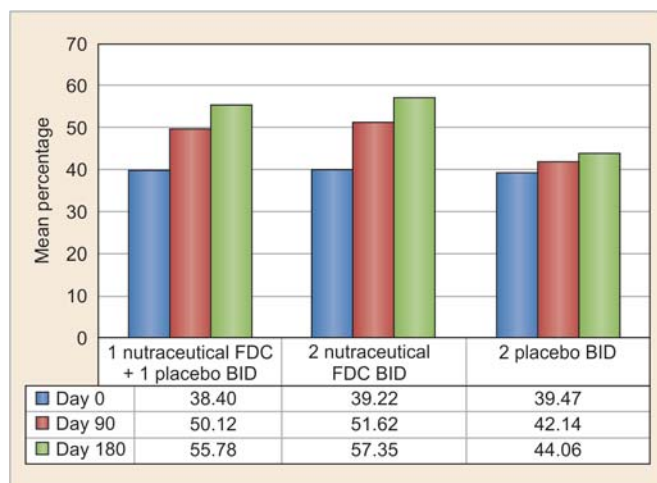


Fig. 3: Motile sperm (%) in each

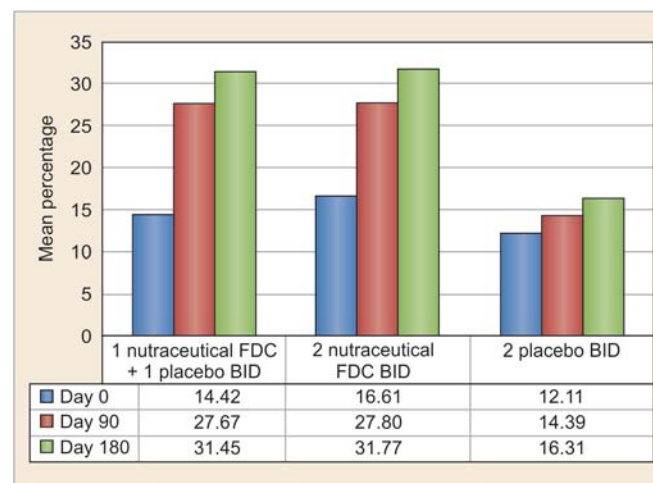


Fig. 4: Sperm with rapid progression WHO A (%)

Table 1: Demographic characteristics of participants

Treatment group	N	Age (years)		Height (cm)		Weight (kg)	
		Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Arm 1	46	32 \pm 4	22-40	166 \pm 7	149-181	70 \pm 13	52-105
Arm 2	43	30 \pm 5	22-45	168 \pm 6	156-185	68 \pm 11	45-99
Arm 3	36	30 \pm 5	22-41	165 \pm 5	156-176	65 \pm 10	48-95
All	125	31 \pm 5	22-45	166 \pm 6	149-185	68 \pm 11	45-105

Table 2: Seminal parameters at days 0, 90 and 180 of treatment

Seminal parameters	Day	Arm 1 2 FDC tablets BID (n = 46)	Arm 2 1 FDC + 1 placebo BID (n = 43)	Arm 3 2 placebo BID (n = 36)	p-value on comparison with day 0 value using ANCOVA test		
					Arm 1	Arm 2	Arm 3
Sperm count (M/ml)	0	14.81 \pm 6.08	14.37 \pm 4.39	12.86 \pm 4.98			
	90	26.35 \pm 8.85	24.88 \pm 7.03	14.89 \pm 5.86	<0.0001	<0.0001	0.0134
	180	33.16 \pm 12.35	31.65 \pm 9.71	15.85 \pm 7.73	<0.0001	<0.0001	0.0233
Motile sperms (%)	0	39.22 \pm 8.99	38.40 \pm 9.54	39.47 \pm 8.20			
	90	51.62 \pm 13.04	50.12 \pm 11.34	42.14 \pm 10.64	<0.0001	<0.0001	0.0381
	180	57.35 \pm 14.61	55.78 \pm 11.94	44.06 \pm 9.53	<0.0001	<0.0001	0.0055
Sperm with rapid progression WHO A (%)	0	16.61 \pm 12.35	14.42 \pm 11.32	12.11 \pm 10.67			
	90	27.80 \pm 15.55	27.67 \pm 14.59	14.39 \pm 12.29	<0.0001	<0.0001	0.0568
	180	31.77 \pm 19.04	31.45 \pm 15.93	16.31 \pm 13.82	<0.0001	<0.0001	0.0005
Sperm with slow progression WHO B (%)	0	12.52 \pm 7.26	16.81 \pm 9.18	17.58 \pm 9.35			
	90	14.42 \pm 8.80	15.00 \pm 7.56	16.64 \pm 8.58	0.0927	0.2022	0.2485
	180	15.70 \pm 8.15	14.93 \pm 8.01	16.57 \pm 7.68	0.0023	0.2324	0.1770
No. of pregnancy		6	7	2			

M: Million; BID: Twice daily; p-value <0.05 indicates significant difference wrt day 0 (baseline)

8 subjects (three in arm 1; one in arm 2 and four in arm 3) were reported.

DISCUSSION

Deleterious effects of reactive oxygen species and other oxidant molecules on sperm motility and membrane integrity have been well documented.^{7,8,37-41} Studies have also reported the reduced total oxyradical scavenging capacity of seminal fluid in infertile men with abnormal semen parameters.^{16,42-44}

Various antioxidants such as CoQ10, L-carnitine, zinc, lycopene, glutathione, etc. have been reported useful in poor semen quality and male infertility.⁴⁵⁻⁵¹ Recently, various double-blind controlled studies have shown effectiveness of individual agents in selected cases of male infertility and

suggested therapeutic usefulness of these therapies.²⁴⁻³⁴ However, so far no trial have evaluated the usefulness of these agents when used in combination and especially in Indian population.

Therefore, the current randomized double-blind study evaluated the commercially available FDC of antioxidant on improvements of seminal parameters and its ensuing effect on resultant pregnancy (Figs 5 and 6).

Current study observed over 100% improvement in sperm count at 180 days over their baseline values in both arms of treatment (Table 2), whereas improvement in sperm count was 23% with placebo treatment. Antioxidants treatment also significantly ($p < 0.0001$) improved the sperm motility during the 6 months of administration. At 90 days, motile sperm were increased by approximately 26% over

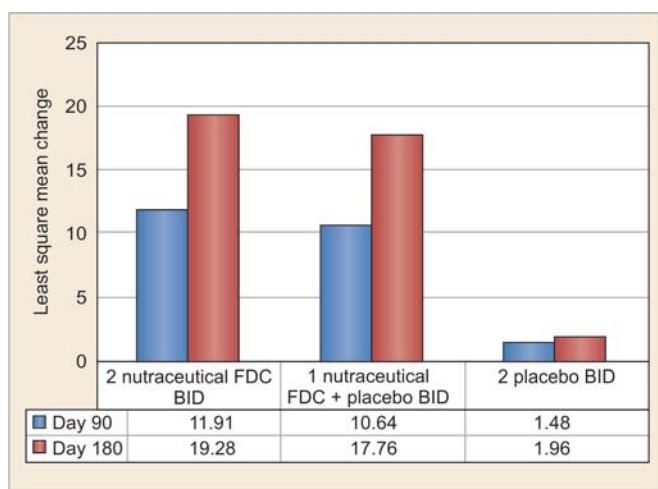


Fig. 5: Improvements in sperm count (million/ml) from baseline

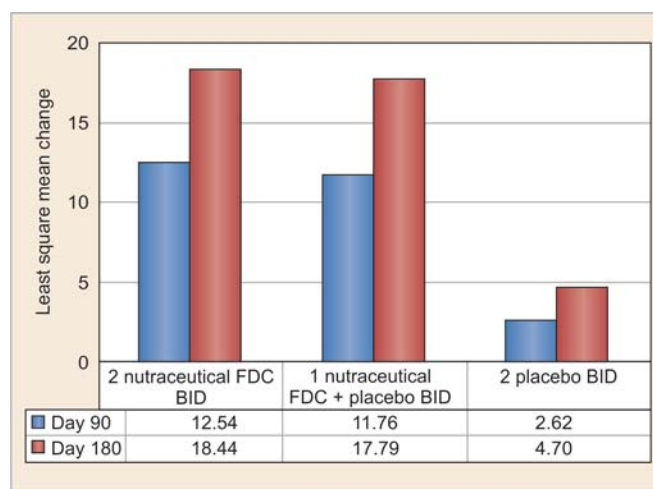


Fig. 6: Improvements in motile sperm (%) from baseline

Table 3: Intergroup analysis on change (LS mean) of seminal parameters from baseline (day 0) to days 90 and 180

Seminal parameter	Day	Treatment arm	N	LS mean ± SE	All	p-value		
						Arm 1 vs 3	Arm 2 vs 3	Arm 1 vs 2
Sperm count (M/ml)	Day 90	Arm 1	46	11.91 ± 1.03	<0.0001	<0.0001	<0.0001	0.3908
		Arm 2	43	10.64 ± 1.06	<0.0001	<0.0001	<0.0001	0.3908
		Arm 3	36	1.48 ± 1.16				
	Day 180	Arm 1	46	19.28 ± 1.58	<0.0001	<0.0001	<0.0001	0.5035
		Arm 2	43	17.76 ± 1.63	<0.0001	<0.0001	<0.0001	0.5035
		Arm 3	36	1.96 ± 1.76				
Motile sperms (%)	Day 90	Arm 1	46	12.54 ± 1.27	<0.0001	<0.0001	0.0003	0.3295
		Arm 2	43	11.76 ± 1.30	<0.0001	<0.0001	0.0003	0.3295
		Arm 3	36	2.62 ± 1.43				
	Day 180	Arm 1	46	18.44 ± 1.56	<0.0001	<0.0001	<0.0001	0.466
		Arm 2	43	17.79 ± 1.62	<0.0001	<0.0001	<0.0001	0.466
		Arm 3	36	4.70 ± 1.73				
Sperm with rapid progression: WHO A (%)	Day 90	Arm 1	46	10.77 ± 1.36	<0.0001	<0.0001	<0.0001	0.7930
		Arm 2	43	11.28 ± 1.39	<0.0001	<0.0001	<0.0001	0.7930
		Arm 3	36	2.21 ± 1.53				
	Day 180	Arm 1	46	15.15 ± 1.66	<0.0001	0.0002	<0.0001	0.7514
		Arm 2	43	15.91 ± 1.72	<0.0001	0.0002	<0.0001	0.7514
		Arm 3	36	5.52 ± 1.86				

N: Number of subjects; LS mean: Least square mean of change from baseline; SE: Standard error; p-value <0.05 indicates significant at 5% level

baseline in both arms of treatment and by 38% in 2 tablets twice-daily arm, and 43% in 1 tablet twice-daily arm at 180 day of treatment as compared to only of 10 and 12% with placebo at days 90 and 180 respectively (Tables 2 and 3).

Three times more pregnancies seen with antioxidant FDC treatment over the placebo treatment in the current study suggests that the positive benefits of therapy on seminal parameters have desired consequent clinical benefits on pregnancy outcome (Table 2).

Though the results of the current study cannot be directly compared with the previous studies on individual agents,²⁴⁻³⁴ however they are in their close confirmation. A direct correlation among these parameters was not proved, a positive dependence was evident with use of Cramer's index of association. The seminal plasma or intracellular variations of CoQ10 have been positively related to the improvement of the kinetic parameters of sperm cells, with a statistically significant dependence.^{15,16}

As recommended in management of male subfertility with antioxidants study observed comparable results with two dosage regimens; i.e. 2 tablets twice daily or 1 tablet twice daily against all primary and secondary efficacy end points. The comparable efficacy of two different dosage regimen observed in the study, is perhaps results of relatively low body weight of participants in this study of Indian population.

The FDC formulation appears to be largely safe, since very few mild adverse events like upper gastrointestinal discomfort reported in treatment groups got self-resolved without discontinuation of therapy and were similar to the type and frequency as of in placebo group. Very low adverse events in current study could be due to demographic variation and dietary habits in India, as approximately 75% of the body stores of L-carnitine are derived from the dietary sources.

The data of this placebo-controlled, double-blind randomized trial confirm a significant improvement of sperm cell kinetic features after 3 and 6 months of administration of fixed dose nutraceutical combination on the basis of both manual semen analysis.

Our future research work should explore how oxidative stress affects subfertility, male reproductive health and how health affects reproduction.

CONCLUSION

The commercially available FDC of antioxidative nutraceuticals of CoQ10, lycopene, L-carnitine and zinc significantly impart therapeutic benefits in oligospermia and asthenozoospermia in male subfertility. The FDC is quite safe and the dosage of 1 tablet twice daily may be used in subjects with lower body weight instead of 2 tablets twice

daily. So far this is the largest RCT on male subfertility on any combinational nutraceutical intervention that highlight the usefulness of antioxidants in oligoasthenozoospermia.

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