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ORIGINAL PAPER

Evaluation of *in vivo* supplementation of 2660 mg D-aspartic acid and 200 mg ubiquinol and 10 mg zinc on different semen parameters in idiopathic male infertility: A randomized double blind placebo controlled study

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Summary

Introduction: About 20-30% of cases of infertility are attributed to male factor and males are also contributing to infertility in a further 20%. Idiopathic male subfertility is the commonest cause in most cases. D-aspartic acid (D-Asp) is an endogenous amino acid occurring in several tissues and cells of both invertebrates and vertebrates.
The current study is one of the first to evaluate the in vivo supplementation of D-Asp in idiopathic male infertility. Thus, we aimed in the current study to evaluate the in vivo effect of D-Asp, zinc and Co-enzyme Q10 (Co-Q10) supplementation on different semen parameters and serum testosterone level in idiopathic male infertility.
Methods: A total of 75 infertile patients were recruited from the outpatient andrology clinic from March 2023 to June 2024. The current study was registered at the UMIN clinical registry trials prior to initiating the study (UMIN000050023). Group (A) included 24 infertile patients who received 2660 mg D-Asp plus 200 mg of ubiquinol plus 10 mg zinc once daily for 3 months. Group (B) included 24 infertile patients who received placebo (starch granules) daily for 3 months.
Results: Interestingly, patients in group (A) who received 2660 mg D-Asp plus 200 mg of ubiquinol plus 10 mg zinc once daily for 3 months showed significant improvement in progressive sperm motility after 3 months (10.63 ± 8.64 vs 15.21 ± 12.11, p = 0.047). Also, they showed highly significant increase in total testosterone level (5.06 ± 1.74 vs 5.89 ± 1.62, p = 0.009).
Conclusions: D-Asp plus ubiquinol plus zinc are promising ingredients that showed good results when administrated once daily to infertile males.

KEY WORDS: *Idiopathic male infertiliy; Total testosterone; Progressive sperm motility; D-aspartic acid; Ubiquinol; Zinc.*

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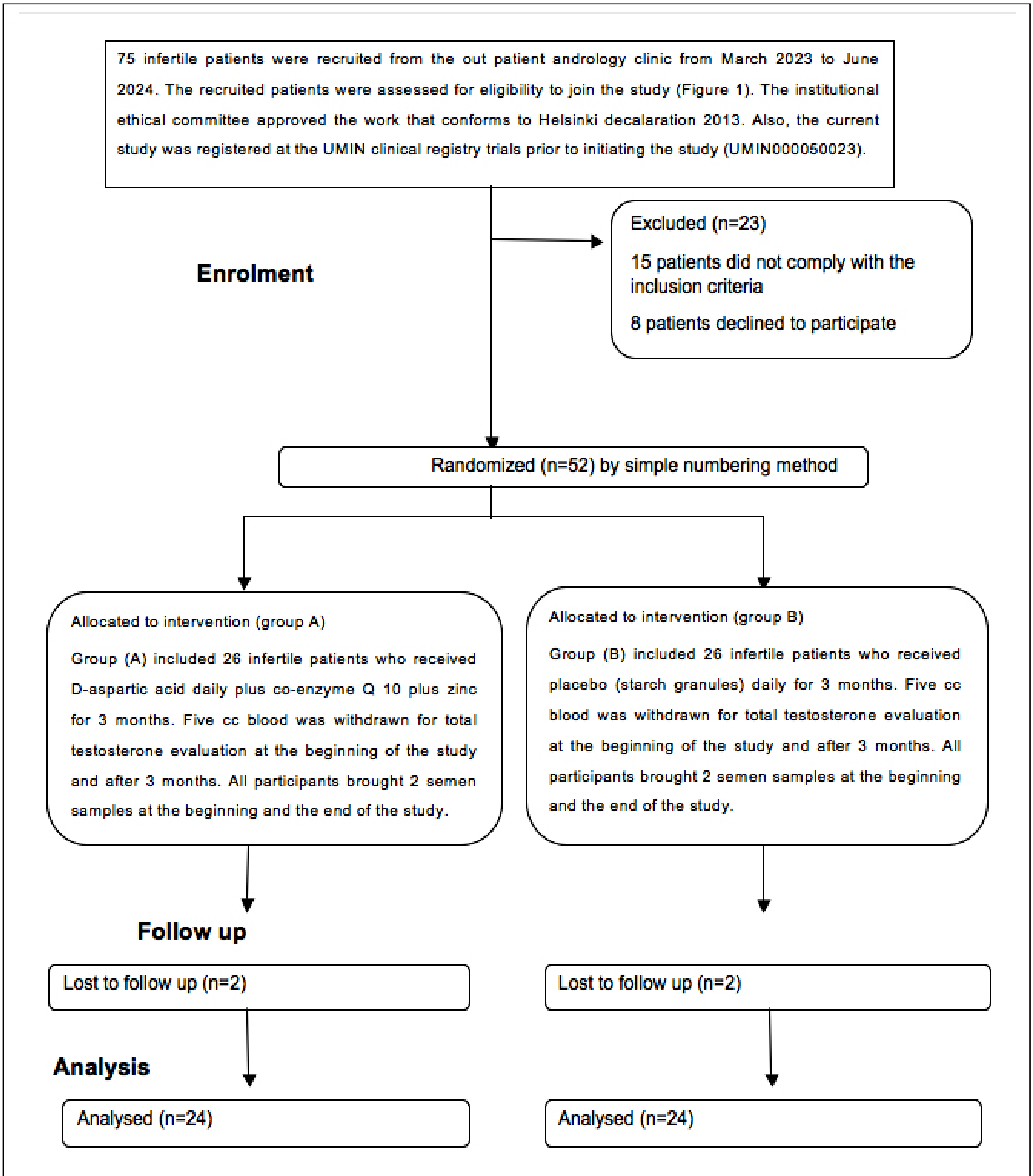
INTRODUCTION

About 20-30% of cases of infertility are attributed to male factor and males are also contributing to infertility in a further 20%. Idiopathic male subfertility is the commonest cause in most cases (1). *D-aspartic acid* (D-Asp) is an endogenous amino acid occurring in several tissues and cells of both invertebrates and vertebrates (2). It was first detected in the brain and optic lobes of the cephalopod mollusy Octopus vulgaris and later in the nervous and endocrine systems of various animal phyla such as crustaceans, amphibians, reptiles, fish, chicken, rat, and man (3-14). *Topo et al.* conducted a clinical trial on 23 healthy male volunteers who consumed 3.12 gram of sodium D-Asp for 12 consecutive days (15). They found significant increase in LH and testosterone levels after 12 days by 33% and 42%, respectively (15). Thus, *Topo et al.* postulated that consumed D-Asp may also be remained in the testis and it continued to stimulate the testosterone production in the testis (15). In the aforementioned study the mean baseline testosterone were within 25% of the lower clinical range (3-10 ng/mL) and D-Asp supplementation elevated testosterone levels to approximately 50% of the clinical range (4.5-6.4 ng/mL) (15). These findings can be explained by the fact that D-Asp acts directly on the pituitary gland inducing an increase of LH releasing (15-16). Furthermore, two *in vitro* studies had demonstrated a beneficial effect of Zn, D-Asp and *Co-enzyme Q10* (Co-Q10) on sperm motility, recovery of spermatozoa by swim-up and lipid peroxidation (17, 18).
On the contrary, *Willoughby et al.* who conducted a study on twenty apparent healthy and heavy resistance-trained men (19). The participants trained 4 times/week while orally taking either 3 gram of placebo or D-Asp in the morning upon waking (19). There was no effect on muscle strength, body mass and serum hormones after 28 days of D-Asp supplementation (19).
To the best of our knowledge, the current study is one of the first to evaluate the *in vivo* supplementation of D-Asp in male infertility.
Thus, we aimed in the current study to evaluate the *in vivo* effect of D-Asp, zinc and Co-Q10 supplementation on different semen parameters and serum testosterone level in idiopathic male infertility.

Materials and methods

A total of 75 infertile patients were recruited from the outpatient andrology clinic from March 2023 to June 2024. The recruited patients were assessed for eligibility to join the study (Figure 1). The institutional ethical committee of Alexandria University approved the study on 23/1/2023 that conforms to Helsinki declaration 2013 (20). Also, the current study was registered at the UMIN clinical registry trials prior to initiating the study (UMIN000050023). Twenty-three patients were excluded from the study. The remaining 52 patients were equally randomized by simple numbering method into 2 groups. Unfortunately, 4 patients dropped out of the study, 2 patients were from group (A) and 2 patients were

Figure 1.
Study flow chart.



from group (B) (Figure 1). Group (A) included 24 infertile patients who received *Spertility®* that consists of 2660 mg D-Asp plus 200 mg of ubiquinol (active form of Co-Q10) plus 10 mg zinc once daily for 3 months. Group (B) included 24 infertile patients who received placebo (starch granules) daily for 3 months. Five cc blood was withdrawn for total testosterone evaluation at the beginning of the study and after 3 months. All participants brought 2 semen samples at the beginning and at the end of the study. Semen analysis was processed according to the 5th guidelines of the WHO (21).

Inclusion criteria of the patients

Any infertile male aged 25 to 40 years old suffering from idiopathic infertility was included.

Exclusion criteria of the patients

Any idiopathic infertile male suffering from varicocele or leukocytospermia or immunological infertility was excluded. Also, any infertile patient with hormonal imbalance was excluded. Finally, any infertile patient with small testicular volume < 8 ml was also excluded.

Results

The current study did not show any significant difference in the mean age of the participants of both groups (31.25 years, ± 7.86; 33.96 years, ± 8.11, respectively, p = 0.062). Interestingly, patients in group (A) who received *Spertility®* showed significant improvement in progressive sperm motility after 3 months of daily supplementation of D-Asp (10.63 %, ± 8.64, 15.21 %, ± 12.11, p = 0.047, respectively) (Table 1).

They also showed significant increase in total testosterone level after 3 months of daily supplementation of D-Asp (5.06 ng/dl, ± 1.74; 5.89 ng/dl, ± 1.62, respectively, p = 0.009) (Table 1).

Conversely, total sperm concentration and sperm motility and abnormal forms did not show any improvement after daily supplementation of D-Asp in patients of group (A) (Table 1). Furthermore, patients in group (B) who received placebo did not show any improvement in total sperm concentration, sperm motility, progressive sperm motility, abnormal forms and total testosterone (Table 2).

Despite, significant improvement in progressive sperm motility and significant increase in total testosterone in patients in group (A), yet, pregnancy rate was 8.3% only. Owing to the fact that only 2 patients out of 24 patients in group (A) succeeded to impregnate their wives.

Statistical methods

Data management and statistical analysis were performed using the *Statistical Package for Social Sciences* (SPSS) version 25. Numerical data were summarized using means and standard deviations or medians and ranges. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Mann-Whitney U and Wilcoxon Signed Ranks Tests were used for the comparison between groups. All p-values are two-sided. P-values ≤ 0.05 were considered significant.

Discussion

The current study had demonstrated significant improvement in progressive sperm motility in group (A) patients who received *Spertility®* daily for 3 months. Similarly, two previous studies demonstrated beneficial effects of *in vitro* supplementation of D-Asp on progressive sperm motility (17, 18). Moreover, an animal study revealed an improvement in sperm function of rabbits after being adminstered D-Asp (22). In the same context, *D'Aniello et al.* (2005) was one of the first to demonstrate lower levels of this amino acid in the semen of patients with oligoastenoteratozoospermia than in fertile men (23). The significant improvement in sperm motility in the current study can also be explained by the presence of zinc and Co-Q10 in the ingredients of *Spertility®*. Several studies had shown the beneficial effects of zinc in male inferility (18, 24-26). In contrats, *Foresta et al.* (2014) failed to demonstrate any correlation between zinc and sperm motility (27).

Table 1.
Changes in semen parameters and total testosterone in group (A) before and after Spertility®.

		Mean	Std deviation	Minimum	Maximum	P-value
Sperm concentration (10^6/ml)	baseline	35.15	± 23.74	9.30	110.20	0. 989
	After 3 months	36.14	± 24.33	6.30	92.80	
Sperm motility %	baseline	45.83	± 10.18	25.00	65.00	0. 441
	After 3 months	44.79	± 8.91	30.00	65.00	
Sperm progressive motility %	baseline	10.63	± 8.64	.00	25.00	0.047
	After 3 months	15.21	± 12.11	.00	60.00	
Abnormal forms %	baseline	61.46	± 11.75	20.00	80.00	0.242
	After 3 months	63.96	± 13.67	20.00	80.00	
Total testosterone (ng/dl)	baseline	5.06	± 1.74	2.56	8.20	0.009
	After 3 months	5.89	± 1.62	3.80	9.50	

Table 2.
Changes in semen parameters and total testosterone in group (B) before and after placebo.

		Mean	Std deviation	Minimum	Maximum	P-value
Sperm concentration (10^6/ml)	baseline	32.75	± 40.22	5.00	201.00	0.255
	After 3 months	31.58	± 39.44	0.00	180.00	
Sperm motility (%)	baseline	37.21	± 23.86	0.00	88.00	0.269
	After 3 months	35.00	± 20.54	0.00	70.00	
Sperm progressive motility (%)	baseline	6.25	± 6.63	0.00	25.00	0.331
	After 3 months	7.08	± 6.24	0.00	20.00	
Abnormal forms (%)	baseline	54.38	± 16.17	20.00	80.00	0.982
	After 3 months	53.96	± 18.33	20.00	98.00	
Total testosterone (ng/dl)	baseline	5.75	± 1.67	3.00	8.80	0.884
	After 3 months	5.82	± 1.77	2.70	9.70	

Additionally, Co-Q10 has a well-established dual action as electron/proton carrier in mitochondrial bioenergetic chain and antioxidant agent (18). In the same context, *Li et al.* (2006) found significant different concentrations of this antioxidant in the seminal plasma of fertile men and infertile patients (28). Conversely, *Nadjarzadeh et al.* (2014) had revealed that this molecule had a potent antioxidant effect (29). Furthermore, *Spertility®* contains ubiquinol which is the active form of Co-Q10 that facilitates its gastro-intestinal absorption. Similarly, *Garrido-Maraver et al.* (2014) demonstrated the effect of different formulations on gastro-intestinal absorption (30). Another interesting finding of the current study was the highly significant increase in total testosterone level in group (A) patients who received Sperility once daily for 3 months. Consistently, a study had reported a steroidogenic role of D-Asp in humans and rats (15).

Quite the reverse, *Willoughby et al.* (2014) failed to demonstrate any change in serum testosterone level in resistance-trained men after being resistance trained 4 times weekly for 1 month and after ingesting 3 g daily of D-Asp (19). It should be mentioned that the dose of D-Asp in the aforementioned study was higher than the concentration of D-Asp in *Spertility®*. Notably, the role of testosterone in male infertility is well established by two important reviews showing the pivotal role of androgen in spermatogensis (31, 32). In the same context, *Carvalho et al.* (2022) had revealed that eugenol administration to Wistar rats reduced serum testosterone and sperm viability (33). On the contrary, a previous study had stated that serum testosterone levels demonstrate no relationship to sperm concentration (34). To wrap up, *Spertility®* administration in the form of once daily sachet for 3 months had shown significant improvement in progressive sperm motility and highly significant increase in serum total testosterone. To the best of our knowledge, the current study is one of the first to demonstrate the impact of *in vivo* supplementation of D-Asp plus ubiquinol plus zinc to infertile males. Despite such achievements that were shown in the current study, yet, the pregnancy rate was very low as two patients only from group (A) succeeded to conceive naturally. This disappointing finding could be attributed to the small sample size and the short period of follow up. Admittedly, there are several limitations of the current study. Firstly, the small sample size and short duration of follow up are seen as major limitations of the current study. Nevertheless, the proper and consistent study design and being a prospective one added strength to the current findings. Also, we were not able to measure luteinizing hormone and sperm DNA fragmentation index adding further limitations of the current study. Finally, we were not able to utilize the 6th edition of the WHO for semen analysis processing (35).

Conclusions

D-Asp plus ubiquinol plus zinc are promising ingredients that showed good results when administrated once daily to infertile males. Future cohort studies that evaluate these ingredients *versus* L-carnitine are needed to affirm these findings.

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Declarations

Ethical approval: *The study was prospectively registered at UMIN under the following serial number 000050023.*

Availability of data and material: *All inquiries can be directed to the corresponding author.*

Competing interests: *The authors declare no competing interests.*

Funding: *This study did not receive any fund.*

Authors' contributions: *SFG, AEN, TH and YE developed the conception and study design; MAA, IM, AZ, MWR, AA, DR and OZ recruited the cases; HS performed the statistics of the study; SFG intellectually drafted the manuscript and critically revised the data. All authors approved the final draft.*
SFG drafted the initial manuscript; SFG revised the article critically; SFG reviewed and edited the article; NN designed the study; SAA performed the lab work of the study; AO, AE, AZ, AAS collected and analyzed the data. All authors reviewed the manuscript.

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