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Abstract

Oligospermia, defined as reduced sperm concentration in the seminal fluid, frequently coexists with disturbances in other seminal parameters, including sperm viability, which reflects the structural and functional integrity of the sperm plasma membrane. L-arginine, a semi-essential amino acid and principal substrate for nitric oxide synthase, contributes to nitric oxide biosynthesis and polyamine metabolism, both implicated in testicular blood flow regulation, germ cell maturation, and sperm functional competence. Although several studies have examined L-arginine in combination with other antioxidants, direct evidence regarding its standalone effect on sperm viability remains limited. This controlled interventional study evaluated the effect of oral L-arginine on sperm viability in men with oligospermia. Twenty men aged 30-50 years with oligospermia were assigned to an intervention group (n=10), receiving oral L-arginine at 4 g/day for 12 weeks, and a control group (n=10) receiving no treatment, under similar monitoring conditions. Sperm viability was assessed using the trypan blue exclusion test and expressed as mean percentage $\pm$ standard deviation, with group comparisons performed using the Mann-Whitney U test and $P < 0.05$ considered statistically significant. The mean percentage of viable sperm was significantly higher in the L-arginine group ($59.98 \pm 3.88\%$) than in the control group ($40.11 \pm 1.36\%$), a difference of 19.87 percentage points that was highly significant ($P < 0.001$). These findings indicate that oral L-arginine supplementation at 4 g/day for 12 weeks significantly improves sperm viability in men with oligospermia and may serve as a promising adjunctive nutritional intervention for male infertility.

Keywords: L-arginine; Oligospermia; Sperm viability; Male infertility

Introduction

Infertility is defined as the inability of a couple to achieve pregnancy after one year of regular, unprotected intercourse. This disorder represents a significant reproductive health concern, with male factors playing a primary or contributing role in approximately half of all cases. Impairments in spermatogenesis, decreased sperm concentration, reduced motility, and abnormal morphological alterations are recognized as the primary determinants associated with male infertility (Agarwal et al., 2021). Oligospermia refers to a reduction in sperm concentration within the seminal fluid. According to the World Health Organization guidelines, the lower reference limit for sperm concentration in fertile men is established at sixteen million spermatozoa per milliliter. Nevertheless, the diagnostic assessment of male infertility should not rely solely on a single parameter, as decreased sperm concentration frequently coexists with disturbances in motility, morphology, plasma membrane integrity, and sperm viability (Cooper et al., 2010).

Sperm viability reflects the functional and structural integrity of the cell membrane. An intact plasma membrane is essential for maintaining ionic homeostasis, cellular energy production, progressive motility, and the execution of physiological cascades necessary for fertilization. Non-viable spermatozoa typically exhibit compromised membrane integrity, which facilitates the influx of vital dyes into the intracellular space. Consequently, dye exclusion assays, such as the trypan blue staining method, are extensively utilized to differentiate viable from non-viable spermatozoa (Cao et al., 2011; Chung et al., 2024). Oxidative stress represents a critical pathophysiological mechanism underlying sperm damage in infertile men. Due to the high proportion of polyunsaturated fatty acids within their plasma membrane and their restricted intracellular antioxidant capacity, spermatozoa are inherently susceptible to reactive oxygen species (ROS). Excessive accumulation of ROS triggers lipid peroxidation of the sperm membrane, mitochondrial dysfunction, impaired motility, DNA fragmentation, and a marked reduction in sperm viability (Abdollahi et al., 2026; Agarwal et al., 2003; Aitken, 2020; Asadi, 2017a, 2017b; Haghani et al., 2018).

L-arginine is a semi-essential amino acid implicated in diverse physiological pathways throughout the body. It serves as the primary obligate substrate for nitric oxide synthase enzymes, contributing directly to the biosynthesis of nitric oxide (NO). Nitric oxide participates in regulating vascular tone, tissue microcirculation, and specific physiological processes associated with male reproductive function. Furthermore, L-arginine acts as a crucial precursor in the polyamine biosynthetic pathway, where downstream metabolites participate in cellular division, male germ cell maturation, chromatin stabilization, and sperm functional competence (Lefèvre et al., 2011; Wu & Morris Jr, 1998). Several clinical investigations have evaluated the therapeutic impact of L-arginine-containing formulations on seminal parameters. In most of these studies, L-arginine was administered concurrently with other pharmacological agents such as L-carnitine, zinc, selenium, coenzyme Q10, and antioxidant vitamins; thus, isolating the independent pharmacological effect of L-arginine remains constrained. However, documented improvements in specific sperm parameters, including concentration and motility, emphasize that evaluating the distinct role of L-arginine in patients with impaired spermatogenesis remains clinically valuable (Sarmidi et al., 2022).

Despite the crucial diagnostic value of sperm viability in determining semen quality, direct clinical evidence regarding the therapeutic efficacy of standalone oral L-arginine administration at a defined dosage over a twelve-week spermatogenic duration, remains scarce. Therefore, the present study was conducted to evaluate the effect of oral L-arginine supplementation at a daily dosage of four grams for twelve consecutive weeks on sperm viability percentage in patients with oligospermia.

Materials and Methods

This study was conducted as a controlled interventional study with two parallel groups. The study population consisted of 20 men with oligospermia, aged 30-50 years, who had visited the relevant center for infertility evaluation and treatment. After assessment according to the inclusion and exclusion criteria, the participants were divided into two groups: a control group and an intervention group. Each group consisted of 10 participants. The inclusion criteria were men aged 30-50 years, a history of infertility, and oligospermia based on semen analysis results. The presence of abnormalities in at least two semen analyses performed four weeks apart, including a sperm concentration of less than 20 million spermatozoa per milliliter, sperm motility of less than 25%, or normal sperm morphology of less than 30%, was considered a laboratory inclusion criterion. Participants were required to be able to take L-arginine regularly for 12 weeks and attend the final assessment. They also had to be willing to comply with the standard requirements for semen sample collection, including 3-4 days of sexual abstinence (Sánchez-Álvarez et al., 2012).

Individuals older than 50 years, those who consumed tobacco or alcohol, and those with grade 3 or 4 clinical varicocele, testicular atrophy, ejaculatory disorders, azoospermia, Sertoli-cell-only syndrome, or a history of major genitourinary surgery were excluded from the study. Individuals with severe endocrine disorders or anatomical abnormalities affecting fertility were also excluded. Additional exclusion criteria included a leukocyte count exceeding 1×10^6 cells/mL of semen or evidence of an active genitourinary tract infection. The use of any medication known to affect spermatogenesis or any supplement related to male infertility within the two months before the beginning of the study was also considered an exclusion criterion. In the intervention group, L-arginine was administered orally at a dose of 4 g/day for 12 consecutive weeks.

Participants in the control group did not receive the study medication during the study period and were followed under similar monitoring conditions. The purpose of comparing the two groups was to evaluate the difference in sperm viability percentage after completion of the intervention period. Semen samples were collected after 3-4 days of sexual abstinence. Samples were obtained by masturbation directly into sterile containers. After collection, the samples were incubated at 37°C under ambient atmospheric conditions, without supplemental carbon dioxide, for 20-30 minutes to allow complete liquefaction. After liquefaction, the samples were prepared for the required laboratory assessments. For the swim-up procedure, the washing and separation of motile spermatozoa were performed according to the laboratory protocol. Sperm counting was performed using a Makler counting chamber (Campbell et al., 2021).

Sperm viability was assessed using the trypan blue exclusion test. In this method, equal volumes of liquefied semen and 0.4% trypan blue solution were mixed. The resulting mixture was examined after approximately 5-10 minutes. A wet smear was prepared, and the sample was evaluated under a light microscope at 400× magnification. Viable spermatozoa with intact cell membranes did not absorb the dye and appeared unstained or lightly stained. In contrast, dead spermatozoa absorbed the blue dye because of compromised cell-membrane integrity (Kandil et al., 2021; Li et al., 2025).

Sperm viability was calculated as the percentage of viable spermatozoa. Direct proportional assessment was performed by microscopic examination of the trypan-blue-stained wet smear according to the following formula:

$$\text{Sperm viability (\%)} = \text{Number of viable spermatozoa} / \text{Total number of counted spermatozoa} \times 100$$

The data were reported using descriptive statistical measures, including the mean and standard deviation (SD). Sperm viability percentages were compared between the control and intervention groups. Data were

analyzed using STATA version 14.0. The results were evaluated using the Mann-Whitney U test. A P-value of less than 0.05 ($P < 0.05$) was considered indicative of statistical significance. The study was conducted in accordance with the principles of medical research ethics and the provisions of the Declaration of Helsinki (Strober, 2015). Before enrollment, the study objectives, sampling procedures, potential benefits, and possible adverse effects of L-arginine administration were explained to the participants. Written informed consent was then obtained from all participants. All information related to the participants was recorded and maintained confidentially.

Results

Effect of L-arginine on sperm viability

In the present study, sperm viability was evaluated and statistically compared between the two experimental groups, with a P-value of less than 0.05 considered indicative of statistical significance. Analysis of the data revealed that the mean percentage of viable sperm was markedly higher in the group receiving L-arginine supplementation (59.98 ± 3.88) than in the control group (40.11 ± 1.36). This substantial improvement demonstrates that oral administration of L-arginine at a dose of 4 g per day, over a period of 12 consecutive weeks, resulted in a significant enhancement in the percentage of viable sperm. The observed difference between the two groups was highly statistically significant ($P < 0.001$), suggesting that L-arginine supplementation may exert a beneficial effect on sperm viability (Figure 1).

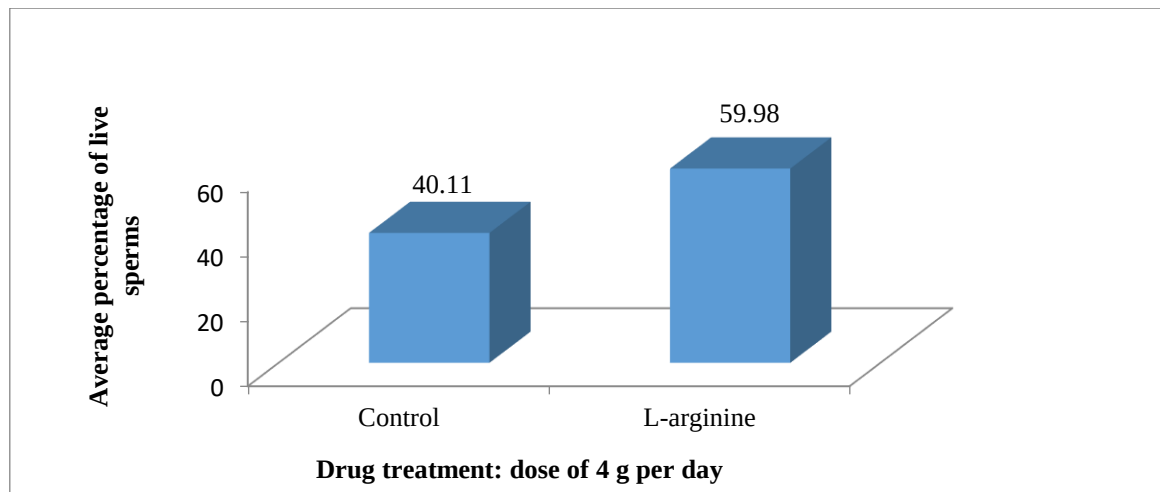


Figure 1. Comparison of sperm viability between the intervention group (L-arginine 4 g per day for 12 consecutive weeks) and the control group.

Discussion

The main finding of this study showed that the percentage of sperm viability in the L-arginine group was higher than that in the control group. The mean sperm viability in the intervention group was $59.98 \pm 3.88\%$ and in the control group was $40.11 \pm 1.36\%$. The difference of 19.87 percentage points between the two groups indicates that oral L-arginine intake was associated with improved sperm viability in men with oligospermia. One possible mechanism for this effect is the role of L-arginine in the nitric oxide production

pathway. L-arginine, as a substrate for nitric oxide synthase, can affect nitric oxide production. Nitric oxide is involved in the regulation of vascular tone and tissue blood flow, and improved testicular blood supply may provide more favorable conditions for the function of Sertoli cells, germ cells, and the process of spermatogenesis (Lefèvre et al., 2011; Wu & Morris Jr, 1998). L-arginine also plays a role in the polyamine metabolism pathway. Polyamines are involved in cellular processes, cell proliferation, germ cell maturation, and the stability of cellular structures. In this regard, increasing the availability of L-arginine may help maintain sperm membrane function by supporting metabolic pathways associated with sperm maturation (Lefèvre et al., 2011).

Oxidative stress may also play a role in explaining the present results. Sperm membranes contain a high content of unsaturated fatty acids. Damage to the membrane is therefore highly sensitive to lipid peroxidation. Oxidative damage to the membrane can cause the entry of vital pigments into the cell and its recognition as dead sperm. Therefore, any reduction in oxidative damage or improvement in the cell's defense capacity could be associated with an increase in the percentage of viable sperm (Agarwal et al., 2003; Aitken, 2020). The present results are in line with some of the previous evidence on the use of nutritional and antioxidant compounds in male infertility. However, it should be noted that many previous studies have examined L-arginine in combination with L-carnitine, zinc, selenium, vitamins, or other supplements. Therefore, the improvements observed in those studies cannot necessarily be attributed to L-arginine alone (Sarmidi et al., 2022).

In the present study, L-arginine was administered at a relatively high dose of 4 g/day for 12 weeks. This distinguishes the present study from studies that have used lower doses or multiple combinations. Clinically, increased sperm viability may be important because adequate viability is essential for maintaining normal sperm motility and function. However, increased percentage viability alone is not sufficient to demonstrate improved fertility. Future studies are needed to assess clinical outcomes such as pregnancy rates, embryo quality, IVF success, and live birth.

Conclusion

In this study, the mean percentage of sperm viability in oligospermic men who received oral L-arginine at a dose of 4 g/day for 12 weeks was higher than in the control group. The observed difference was reported to be statistically significant ($P < 0.001$). This finding suggests that L-arginine may be used as an adjunctive nutritional intervention to improve sperm viability. However, randomized trials with larger sample sizes, placebo groups, adequate blinding, and simultaneous assessment of other sperm quality indicators are necessary to confirm this result.

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