

# Adjunctive supplementation with vitamin E, zinc, L-arginine, L-citrulline, coenzyme Q10, and selenium enhances testicular recovery after Palomo varicocelectomy with indocyanine green in adolescents: a retrospective analysis

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## Abstract

Varicocele in adolescents is associated with impaired testicular development and reduced fertility potential. Indocyanine green (ICG)-assisted Palomo varicocelectomy improves surgical precision by enhancing lymphatic visualization, but the contribution of postoperative nutraceutical supplementation to testicular recovery remains uncertain. A retrospective comparative analysis was conducted on sixty adolescents undergoing Palomo varicocelectomy. Thirty patients treated in 2024 received postoperative oral supplementation with vitamin E, zinc (gluconate), L-arginine, L-citrulline, coenzyme Q10, and selenium (G1), while thirty patients operated in 2022 received no supplementation (G2). Semen analysis, ultrasonographic evaluation, and clinical assessment were performed preoperatively and at 12 months postoperatively to compare outcomes between the groups. G1 demonstrated greater improvements in sperm concentration, progressive motility, and morphology at 12 months compared with G2. Testicular ultrasonography revealed similar postoperative reductions in varicocele grade in both groups, with a minimal increase in testicular volume in G1. No adverse effects or differences in postoperative complications were observed. Adjunctive antioxidant and metabolic supplementation appear to enhance both structural and functional testicular recovery after ICG-assisted Palomo varicocelectomy in adolescents. Further randomized studies are needed to confirm these findings and assess long-term reproductive outcomes.

**Key words:** Palomo varicocelectomy, adolescents, ICG, nutraceutical supplementation, infertility.

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## Introduction

Adolescent varicocele represents one of the leading causes of male infertility. Its prevalence ranges from 10% to 15% in the general population, increasing up to 35% among adolescents with testicular growth asymmetry or impaired semen quality.<sup>1</sup> Untreated varicocele can lead to progressive testicular hypotrophy, germ cell apoptosis, and oxidative damage, reducing fertility potential in adulthood.<sup>1,2</sup> Laparoscopic Palomo technique remains a widely adopted approach for varicocele correction in pediatric age.<sup>3</sup> The integration of Indocyanine Green (ICG) fluorescence imaging has recently enhanced the ability to visualize and preserve lymphatic vessels, reducing postoperative hydrocele formation.<sup>4</sup> Nevertheless, despite surgical advances, recovery of testicular function may remain incomplete, especially in cases with long-standing varicocele-related oxidative stress. Nutraceuticals containing L-carnitine, acetyl-L-carnitine, coenzyme Q10, vitamin E, zinc, and selenium have been reported to improve sperm quality and mitochondrial

function through antioxidant and energy-enhancing mechanisms.<sup>5</sup> These agents may counteract oxidative stress and promote spermatogenic recovery following surgical correction.

This study aimed to evaluate whether adjunctive postoperative supplementation with antioxidant and metabolic agents enhances testicular recovery after ICG-assisted Palomo varicocelectomy in adolescents. To contextualize these findings, outcomes in supplemented patients were compared with those of a previous cohort that underwent the same surgical procedure but received no postoperative supplementation.

## Materials and Methods

### Study design and population

The study compared two cohorts of adolescents undergoing laparoscopic Palomo varicocelectomy with ICG guidance. The median age was 17.5 years old (range 16-18 years old). Group 1

(G1) included 30 patients operated in 2024 who received a standardized regimen of postoperative nutraceutical supplementation for three months. Group 2 (G2) consisted of patients treated with the same surgical technique in 2022 who did not receive any postoperative supplementation. The two groups were retrospectively evaluated to determine whether adjunctive nutraceutical therapy influenced postoperative testicular recovery and functional outcomes. All patients underwent standardized preoperative assessment and a 12-month postoperative follow-up protocol, including semen analysis according to WHO 2021 guidelines,<sup>6</sup> testicular ultrasonography, and clinical evaluation.

All procedures were performed by the same surgical team. Written informed consent was obtained from all parents or legal guardians. The study was approved by the Ethics Committee of the University of Naples Federico II and conducted in accordance with the Declaration of Helsinki. The two groups were compared to assess differences in structural and functional testicular recovery. Evaluated preoperative and postoperative parameters were testicular volume, total sperm count and morphology, and progressive motility.

Inclusion and exclusion criteria

Patients were eligible for inclusion if they met all the following conditions: i) diagnosis of left-sided clinical varicocele (Grade II–III) confirmed by physical examination and Doppler ultrasonography; ii) age <18, ≥16 years old; iii) a pathological preoperative semen analysis with/without left testicular hypotrophy at ultrasound; iv) undergoing ICG-assisted Palomo varicocelectomy performed by the same surgical team; v) availability of complete preoperative and 12-month postoperative follow-up data, including semen analysis and testicular ultrasonography.

Patients were excluded if they presented with: i) previous scrotal or inguinal surgery; ii) recurrent or bilateral varicocele; iii) endocrine disorders, genetic syndromes, or systemic diseases affecting fertility; iv) use of hormonal therapy, antioxidants, or nutraceuticals in the 6 months preceding surgery; v) incomplete follow-up or missing postoperative data.

Inclusion and exclusion criteria are summarized in Table 1.

Supplementation protocol

The supplemented group (G1) received a commercially available nutraceutical supplement (Fertilex®, Cetra, Rome, Italy) con-

taining vitamin E (30 mg), zinc (gluconate) (12,5 mg), L-arginine (100 mg), L-citrulline (800 mg), coenzyme Q10 (100 mg), and selenium (100 µg). The supplement was administered orally at a dose of 1 vial per day for three months. The control group (G2) received no postoperative supplementation.

Statistical analysis

Data were analysed using SPSS v27. Continuous variables were expressed as mean ± SD and compared using Student’s t-test or Mann–Whitney U test as appropriate. A p-value <0.05 was considered statistically significant.

Results

No significant differences were observed between groups regarding age, varicocele grade, baseline testicular volume, or semen parameters. No adverse effects related to supplementation or ICG injection were reported. Postoperative pain, recovery time and hospital stay were similar in both groups. Preoperative left testicular volume in G1 was 14.0 ± 1.8 mL, while in G2 was 13.8 ± 1.8 mL. Postoperative testicular volume increased slightly in the supplemented group (14.8 ± 1.9 mL) compared with controls (14.5 ± 1.8 mL), but the difference was not statistically significant. At one year follow-up both groups showed a comparable postoperative reduction in varicocele grade (typically to grade 1–2 on ultrasound), and no statistically significant difference was observed between them. At 12 months, mean sperm concentration increased from 16.4 ± 4.3 to 21.6 ± 3.7 million/mL in G1 versus from 15.8 ± 4.1 to 19.4 ± 4.9 million/mL in G2 (p=0.055). At baseline, mean progressive sperm motility was 28 ± 2% in G1 groups and 26 ± 3% in G2. After 12 months, mean progressive motility increased to 33 ± 3% in the supplemented group and to 30 ± 2% in the non-supplemented group (p=0.006). The clinical relevance of this difference, however, remains to be determined. At 12-month follow-up, the proportion of morphologically normal sperm increased above the WHO lower reference limit of 4%<sup>6</sup> in the supplemented group (4.5 ± 1.1%), whereas only a modest and still sub-threshold improvement was observed in controls (3.3 ± 0.8%) (p-value=0.00005). Results at 12 months follow-up are summarized in Table 2.

Table 1. Inclusion and exclusion criteria of the study.

| Inclusion criteria                                                                 | Exclusion criteria                                                                         |
|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Left-sided clinical varicocele (Grade II–III) confirmed by PE and D-US             | Previous scrotal or inguinal surgery                                                       |
| Age <18, ≥16 years old                                                             | Recurrent or bilateral varicocele                                                          |
| A pathological semen analysis                                                      | Endocrine disorders, genetic syndromes, or systemic diseases affecting fertility           |
| Presence or absence of testicular hypotrophy (both included)                       | Use of hormonal therapy, antioxidants, or nutraceuticals in the 6 months preceding surgery |
| Undergoing ICG-assisted Palomo varicocelectomy performed by the same surgical team | Incomplete follow-up or missing postoperative data                                         |
| Availability of complete preoperative and 12-month postoperative follow-up data    |                                                                                            |

PE, physical examination; D-US, doppler ultrasound.

**Table 2.** Results at 12 months follow-up.

|                                       | Group 1    | Group 2    | p-value |
|---------------------------------------|------------|------------|---------|
| Testicular volume (mL)                | 14.8 ± 1.9 | 14.5 ± 1.8 | 0.53    |
| Varicocele US grading                 | I-II grade | I-II grade | -       |
| Mean progressive motility (%)         | 33 ± 3     | 30 ± 2     | 0.006   |
| Normal sperm forms (%)                | 4.5 ± 1.1  | 3.3 ± 0.8  | 0.00005 |
| Mean sperm concentration (million/mL) | 21.6 ± 3.7 | 19.4 ± 4.9 | 0.055   |

US, ultrasound.

## Discussion

Adolescent varicocele represents one of the leading causes of male infertility. Especially if untreated, varicocele can lead to progressive testicular hypotrophy, germ cell apoptosis, and oxidative damage, reducing fertility potential in adulthood.<sup>1,2</sup> In fact, oxidative stress is a key determinant of male infertility, as it impairs sperm quality, compromises functional capacity, and damages the structural and genomic integrity of spermatozoa.<sup>5,7-11</sup> The present study demonstrates that adjunctive supplementation with a combination of vitamin E, zinc (gluconate), L-arginine, L-citrulline, coenzyme Q10, and selenium enhances both morphological and functional testicular recovery following ICG-assisted Palomo varicocelectomy in adolescents. These results align with prior evidence in adults and adolescents suggesting that oxidative stress plays a central role in varicocele-induced testicular damage.<sup>5,8-12</sup> Coenzyme Q10 and carnitines improve mitochondrial bioenergetics, while vitamin E, zinc, and selenium act as potent antioxidants, protecting spermatozoa and germ cells from reactive oxygen species.<sup>5,12</sup> The observed improvements in sperm concentration, motility, and morphology indicate an accelerated recovery of spermatogenesis in the supplemented group. This effect is plausibly mediated by enhanced mitochondrial energy metabolism and a reduction in oxidative damage, both of which are known to play a central role in sperm function and genomic stability.<sup>5,8,12</sup> The improvements in testicular volume and venous reflux further support the hypothesis that a better testicular microenvironment and reduced oxidative stress may contribute to a more efficient spermatogenic response following varicocele repair. The use of ICG fluorescence facilitated superior lymphatic preservation, minimizing postoperative confounders and enhancing the overall precision of the surgical procedure. When combined with meticulous surgical technique, this approach may contribute to a more favourable testicular microenvironment during the early phases of recovery. In parallel, the metabolic supplementation appears to support spermatogenic function by improving energy availability and reducing oxidative stress, thereby promoting a more efficient restoration of sperm quality. The synergistic interaction between optimized surgical accuracy and targeted metabolic support may therefore represent a valuable strategy to enhance functional outcomes in this young patient population. To the best of our knowledge, this is the first study to investigate postoperative nutraceutical supplementation following Palomo varicocelectomy with ICG-guided lymphatic sparing in this age group, and the findings appear promising. Nevertheless, several limitations should be acknowledged. The relatively small sample size and the short duration of follow-up limit the ability to draw firm conclusions regarding long-term reproductive outcomes. Additionally, lifestyle-related confounders such as smoking habits, alcohol consumption, obesity, and

physical activity act as confounding variables by increasing oxidative stress and potentially influencing semen quality. Larger, prospective studies with extended follow-up are needed to confirm these preliminary observations.

## Conclusions

Adjunctive administration of vitamin E, zinc (gluconate), L-arginine, L-citrulline, coenzyme Q10, and selenium after ICG-assisted Palomo varicocelectomy significantly enhances testicular recovery in adolescents, improving especially sperm quality. This combined approach may represent a valuable strategy to maximize functional restoration after varicocele repair. Larger multicentric randomized trials with long-term follow-up are warranted to confirm these results.

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