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Positive Effects of a Resveratrol-Based Nutraceutical in Association with Surgical Scleroembolization: A Pilot Retrospective Clinical Trial

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Abstract: Background: Varicocele still today represents a common cause of infertility in young men. The treatment strategy remains a surgical approach such as scleroembolization; however, the complete restoration of spermatic parameters afterward requires an average of six or more months to fully regain optimal seminal parameters. Recently, many studies have demonstrated the beneficial effects of Resveratrol in male fertility, given its potential anti-inflammatory, antiapoptotic, and mitochondrial effects. Therefore, Resveratrol-based nutraceuticals could be promising as an adjuvant to mitigate subfertility in patients with varicocele. **Methods:** In the present study, we retrospectively analyzed the effects of the administration of a Resveratrol-based nutraceutical after the scleroembolization procedure. The improvement of sperm quality in terms of number, motility, and morphology were considered to be the study's main endpoints. A spreadsheet program was used for data analysis, and a p-value of <0.05 was considered significant. **Results:** We found a statistically significant improvement in the spermatic parameters (sperm count and total motility) and an increase in normal sperm after only 4 months of treatment. The supplementation with a Resveratrol-based nutraceutical associated with the surgical procedure showed encouraging results if compared to data from a control group and the results reported in the literature linked to scleroembolization practice alone. In fact, there was a clear improvement in the seminal parameters at 4 months. **Conclusions:** This suggests the positive impact of the Resveratrol-based nutraceutical in synergizing with scleroembolization in reducing the time needed to fully recover sperm function.

Keywords: varicocele; scleroembolization; Resveratrol; infertility

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1. Introduction

Varicocele represents the most common correctable cause of infertility in young men [1]. A varicocele is clinically defined as an abnormal dilation and enlargement of the scrotal venous pampiniform plexus. The incidence of varicocele is approximately 15–20% when considering the entire male population but increases to 40% when considering infertile males [2], indicating a strong connection between clinically significant varicocele and male infertility. Indeed, although not usually accompanied by painful symptoms, varicoceles are clinically significant, as they are the most commonly identified cause of abnormal semen analysis, including low sperm count, reduced sperm motility, and increased abnormal sperm morphology. However, the specific mechanism by which varicocele affects sperm production, structure, and function remains uncertain, despite several theories.

As depicted in the EAU Guidelines for "Sexual and Reproductive Health" [3], the treatment of choice remains a surgical strategy for patients with clinical varicocele and

altered semen parameters. To date, many surgical methods are available for the treatment of varicocele, such as scleroembolization and microsurgical subinguinal varicocelectomy. No clear data indicate the best surgical treatment, but we know that it could improve the symptoms and sperm parameters, offering recurrence rates of less than 4%. It is well known that varicocele repair significantly improves both natural and in vitro fertilization procedures and live birth and pregnancy rates, as well as sperm count, total and progressive motility, morphology, and DNA fragmentation rates. In general, after varicocelectomy, up to 70% of patients have improved sperm quality, with an average of a minimum of two spermatogenic cycles [4] and spontaneous pregnancy occurring between 6 and 12 months [5]. However, the degree of restoration of the seminal parameters and fertility after varicocelectomy is known to vary according to the ages of patients. Specifically, adolescents between 15 and 19 years of age have a complete recovery of the ability to conceive, with a 100% pregnancy rate within the first year of marriage [6], while improvement in the seminal parameters may include only 50% of adults (>19 years of age) undergoing varicocelectomy [7].

Several studies in the literature have previously described the role of inflammation in the pathogenesis of varicocele by describing the state of testicular and sperm inflammation in animal models, particularly in mice, as well as in men with varicocele, with or without alterations in the seminal parameters [5]. In this context, according to the varicocele pathophysiology, a group of cytosolic receptors called nucleotide oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes may be also involved in this mechanism [8]. It is worth noting that, from a mechanistic point of view, oxidative stress and testicular apoptosis are also believed to play significant roles [9].

Recently, both clinical [10–12] and preclinical [13–15] studies have demonstrated that botanical derivatives like Resveratrol (trans-3,5,4′-trihydroxystilbene), a naturally occurring polyphenolic molecule found in several plants, such as *Polygonum cuspidatum* and the seeds of grapes, peanuts, blueberries, bilberries, and cranberries, can have a positive effect on sperm quality and can be useful in numerous chronic pathologies. Indeed, several in vivo and in vitro studies have demonstrated that this compound has anti-inflammatory, antioxidant [16,17], and antiapoptotic properties [18].

From a mechanistic point of view, these actions depend on different pathways; among them, Resveratrol is involved in the activation of antioxidant enzymes, such as catalase and superoxide dismutase, having a crucial role in the lipid damage of human spermatozoa [19]. Furthermore, Resveratrol shows an anti-inflammatory activity by the inhibition of COX2 and NF-KB [20] and protects cells from DNA damage and apoptosis by modulating anti- and proapoptotic mediators, leading to an increase in sperm vitality, sperm DNA integrity, and an improvement in oxidative stress [21].

Moreover, Resveratrol can improve mitochondrial activity and trigger a series of molecular mediators able to affect cellular metabolic mechanisms [22]. Resveratrol also plays a role in calcium signaling cascades and in modulating the mitochondrial intrinsic apoptotic pathway.

This in vitro evidence is corroborated by in vivo preclinical studies on rat models, highlighting the promising use of Resveratrol as a nutraceutical substance adjuvant to mitigate subfertility in varicocele [8,21,23]. As an example, Mendes et al. demonstrated that Resveratrol partially reduces testicular apoptosis and reverses alterations in the sperm motility and promotes mitochondrial activity and DNA integrity [23], as well as improves the oxidative status and sperm vitality [21]. According to the obtained results, Hajipour et al. [8] suggested that Resveratrol might be an adjuvant therapeutic option in patients with varicocele by virtue of decreasing inflammatory events and apoptosis in varicocele-induced rats.

In recent years, only preliminary human clinical research has indicated that nutritional interventions based on Resveratrol can significantly counteract male infertility [10]. All these data suggest the possibility of improving varicocele clinical manifestations and recovery of the seminal parameters by reducing oxidative, inflammatory, and proapoptotic stimuli.

Moreover, it corroborates the potential use of Resveratrol in managing impaired male fertility and varicocele (Figure 1) and suggests the need for extensive studies in this regard.

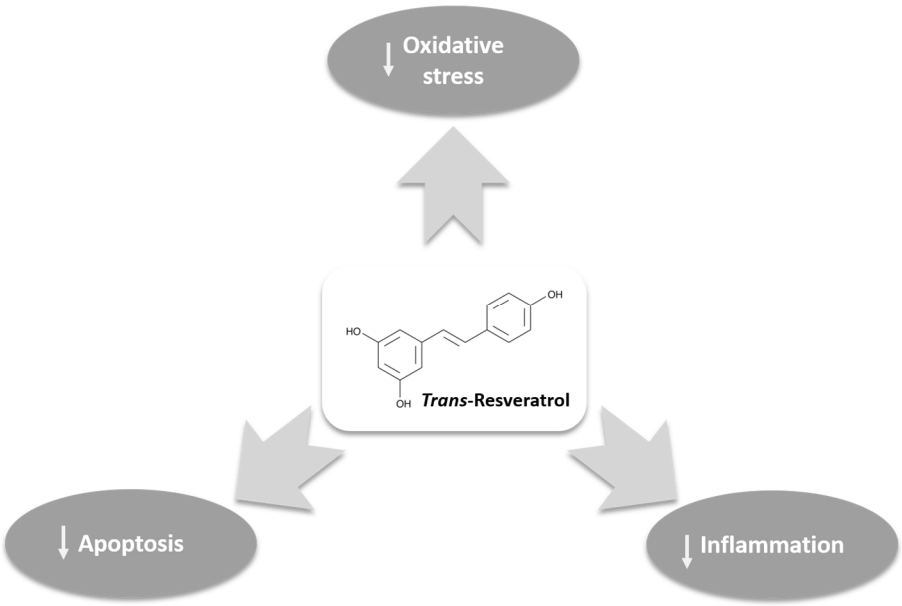

Figure 1. Mechanism of action of Resveratrol in the management of varicocele.

In this context, in this retrospective study, the effects of a nutraceutical containing Resveratrol and magnesium dihydroxide (trademark Revifast®), vitamin B12, vitamin B6, vitamin D, and folic acid were evaluated on the spermatic function after surgical scleroembolization.

The purpose of this study is to investigate whether Resveratrol-based nutraceutical therapy in association with varicocele repair can improve the clinical practice in terms of timing and extent of recovery of the seminal parameters and what impact it may have on fertility.

2. Materials and Methods

This retrospective, controlled study was conducted at the Reproduction Center "Genesi" in association with "Villa Sofia-Cervello" Hospital of Palermo between 2021 and 2023. Patients were admitted to our institution with a diagnosis of bilateral or left-side varicocele and treated with transcatheter percutaneous scleroembolization or through the basilic vein or the right femoral vein.

As the normal clinical practice, antibiotic therapy with 4–5 days of I generation cephalosporin was suggested at discharge. Patients eligible for analysis in the study group were chosen from those who received daily supplementation with 150 mg of Resveratrol and magnesium dihydroxide (trademark Revifast®, Prolabin&Tefarm, Perugia, Italy), 2.5 µg of vitamin B12, 1.4 mg of vitamin B6, 25 µg of vitamin D, and 400 µg of folic acid (two tabs of nutraceutical/day for 4 months), while control patients were chosen between those who did not receive any nutraceutical supplementation.

Inclusion criteria: According to the registry of men submitted to scleroembolization, men with grade II or grade III bilateral and left-side varicocele on physical evaluation after standing for 5 min [24] were considered potentially eligible for the retrospective study, which was restricted to patients seeking consultation at the included center.

Exclusion criteria: According to clinical guidelines, men with known causes for male subfertility other than varicocele, such as cryptorchidism, treated cancer, surgery of the scrotum and of the genital tract, or hypogonadotropic hypogonadism, were excluded from varicocele repair. Azoospermic men were also excluded from the study.

This retrospective analysis was restricted to patients preliminary submitted in our institution to CDU to confirm a bilateral or left-side varicocele by the occurrence of a

continuous left SVR before undergoing a retrograde or anterograde phlebography of the internal spermatic vein and scleroembolization. Clinical examination of the genitals, CDU, and laboratory tests were performed at baseline and at 4 months after the surgical procedure. In total, data from 86 patients (mean age 30.12 ± 7.21) were collected for the study group and 20 patients (mean age 26.49 ± 5.92) for the control group.

Variables and outcomes: Patients' data at baseline were complete with an anthropometric assessment, including weight, height, and body mass index (BMI). As the usual clinical practice, blood samples were collected in the early morning for determination of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and prolactin. Semen analyses were performed in our institutions according to the WHO criteria (2010) [25], and ejaculates were collected through masturbation in both centers by a certified seminologist after 3–5 days of sexual abstinence. All participants considered for the statistical analysis (N = 56) had at least two semen analyses at baseline and at 4 months, provided that no fever, no genital tract infection, and no major trauma or surgical procedures occurred between the two examinations. As the normal clinical practice, following the EAU Guidelines [3], if the semen analysis was abnormal according to the WHO criteria, a second analysis was performed (reporting the result as the mean ± SD). The semen parameters included the total spermatozoa (10^6), total sperm motility (%), forward sperm motility (%), non-forward sperm motility (%), and normal sperm morphology (%).

The primary study outcome was the improvement of sperm quality in terms of number, motility, and morphology. The secondary outcomes obtained were pregnancy (%) and the resolution of SVR after repair.

Scrotal ultrasonography: All patients underwent an evaluation with EchoColor-Doppler both before the intervention and after 3–4 months during the follow-up.

Varicocele repair: The surgical procedure we used to treat varicocele was transcatheter percutaneous scleroembolization, a radiologic technique [26], in which the access employed is by the right common femoral vein after local anesthesia or through the basilic vein.

After a limited fluoroscopy to minimize radiation exposure to patients, the spermatic venous incompetence at its origin from the renal vein was confirmed. The catheter was inserted in the spermatic vein and the tip placed between the distal internal spermatic vein and the pampiniform plexus. The reflux in the pampiniform plexus was verified by fluoroscopy during the Valsalva maneuver, with a wide visualization of the pampiniform plexus. After protection of the testis by hand pressure on the left pampiniform plexus, we used from 1 to 3 vials of lauromacrogol 400 (Atossiclerol 3%).

Post-procedure care: As the usual clinical practice, patients were observed for 1 h after the procedure before discharge. They were advised to rest for 72 h, avoiding Valsalva pressure. After 30 days, the success of the procedure was determined by clinical examination and CDU recording of a retrograde flow. We deemed that the scleroembolization procedure was effective if the complete absence of reflux in the spermatic vein was recorded by CDU in the standing position after varicocelectomy. CDU was repeated after 3 months to confirm the results.

Statistical analysis: A spreadsheet program was used for data analysis. All continuous variables were examined for normality with the D'Agostino–Pearson test. Normally distributed variables were expressed as the mean ± standard deviation (SD). The *t*-test or the Mann–Whitney test was used for comparison as appropriate. Qualitative variables were expressed as the number and proportions and were compared to the chi-square test or Fisher's exact test as appropriate. All the collected variables and outcomes were compared between the baseline and T_4 in a bivariate analysis.

3. Results

Retrospective data were collected from 86 patients relative to the period between 2021 and 2023 in two Sicilian andrological or reproductive centers. The demographic and clinical characteristics of the included patients are shown in Table 1.

Table 1. Demographic data of the study patients at baseline.

Parameters	Study Group
Age	30.12 ± 7.21
Height (cm)	174.72 ± 4.27
Weight (Kg)	79.69 ± 7.77
BMI (Kg/m^2)	26.09 ± 2.24
Smoking (%)	37.5% (yes) 62.5% (no)
Varicocele Dubin (1–3) (%)	54.65% (Degree 2) 45.35% (Degree 3)
Anatomic variants (%)	13.33% (yes) 86.67% (no)
Bilateral varicocele (%)	4.44%
Left-side varicocele (%)	94.44%
Right-side varicocele (%)	1.11%
Intraoperative adverse events (iAEs) (%) *	1.11%
Coil (%)	5.56%
Glue (%)	37.97%
LH	4.01 ± 1.40
FSH	4.46 ± 2.24
Prolactin	12.34 ± 16.63
Testosterone (T)	5.22 ± 3.48
Sperm Total Number ($\times 10^6$)	18.03 ± 10.59
Sperm Total Motility	17.91 ± 9.29
Forward Motility	12.93 ± 13.71
Non-Forward Motility	6.84 ± 7.31
Immobility	79.12 ± 16.42
Normal Morphology	3.92 ± 2.04
Leucocytes < 1 mld (%)	7.78%

Data are reported as *n* (%) or mean ± SD, as appropriate. * iAE: swelling of the inguinal canal, pain, and of embolism.

The laboratory assessment after 4 months of post-surgical treatment with a nutraceutical containing Resveratrol and magnesium dihydroxide (trademark Revifast®), vitamin B12, vitamin B6, vitamin D, and folic acid (two tabs/day for 4 months) showed a statistically significant improvement in the total sperm count (32.30 ± 20.60 × 10^6/ejaculate vs. baseline 18.03 ± 10.59 × 10^6/ejaculate, 91% average increase), total motility (17.91 ± 9.29 × 10^6/ejaculate vs. 31.75 ± 16.34 × 10^6/ejaculate, 75% average increase), and an increase in normal morphology (p = 0.000000242, 64.6% average increase) (Table 2 and Figure 2). These results can be compared to retrospective data collected from 20 control patients with Dubin varicocele II or III who did not receive post-surgical nutraceutical treatment (baseline demographic data in Supplementary Table S1). Table 3 shows a non-significant increase in the total sperm count (mean increase 12%, p = 0.43), total sperm motility (mean increase of 15%, p = 0.43), and normal morphology (mean increase of 15%, p = 0.39) after 4 months post-scleroembolization. To be noted, these mean increases are markedly smaller than the significant increases observed in the study group treated with the Resveratrol-based nutraceutical (Table 2). In the study group, a significant % resolution of varicocele detected by Doppler post-scleroembolization (95%) was observed, as well

as an overall spermiogram improvement (82%) and spontaneous pregnancies (16%) at 6 months after scleroembolization (Table 4). The pregnancy rate at 6 months was 16%, considering the complete study group, but in only 17 patients was this desired, leading to a 70% pregnancy rate among the desired pregnancy group (Table 4). Compared to the control patients, the Resveratrol treatment led to a significant number of patients in which the overall spermiogram improved (82% study group vs. 50% control group, $p = 0.00166$) (Table 4).

Table 2. Outcome comparison of the study group at baseline and 4 months.

Outcome	Baseline Study Group (n = 56)	4 months Study Group (n = 56)	Average Increase %	p
Sperm Total Number ($\times 10^6$)	18.03 ± 10.59	32.30 ± 20.60	91%	0.000011
Sperm Total Motility	17.91 ± 9.29	31.75 ± 16.34	75%	0.000000296
Normal Morphology	3.92 ± 2.04	6.45 ± 2.80	64.6%	0.000000242

Data are reported as the average increase (%) or mean ± SD, as appropriate.

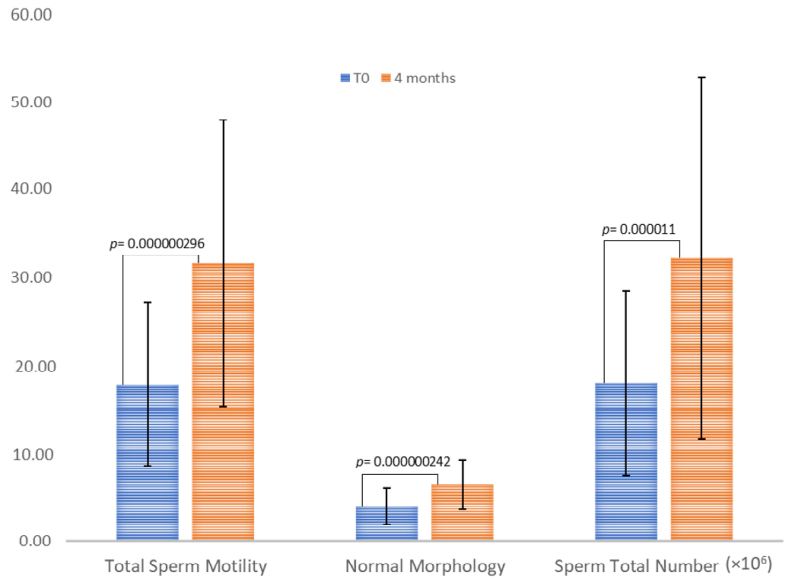

Figure 2. Improvements of the semen parameters at 4 months. Data are reported as the mean ± SD.

Table 3. Outcome comparison of the control group at baseline and 4 months.

Outcome	Baseline Control Group (n = 20)	4 months Control Group (n = 20)	Average Increase %	p
Sperm Total Number ($\times 10^6$)	16.45 ± 8.38	19.50 ± 9.81	12%	0.43
Sperm Total Motility	38.00 ± 17.85	42.37 ± 16.40	15%	0.43
Normal Morphology	61.69 ± 31.73	70.79 ± 23.90	15%	0.39

Data are reported as the average increase (%) or mean ± SD, as appropriate.

Table 4. Improvements (%) and outcomes after scleroembolization and treatment with the nutraceutical.

Outcome	(*n* = 86)	(*n* = 20)	*p*
Resolution of varicocele detected by Doppler post-scleroembolization *n* (%) [1]	82 (95%)	18 (90%)	0.6926
Improvement of spermiogram *n* (%) [1]	70 (82%)	10 (50%)	0.00166
Spontaneous pregnancy *n* (%) [2]	14 (16% on total number) (70%; desired *n* = 17)	1 (5% on total) (20%; desired *n* = 5)	0.13230

Data are reported as *n* (%). [1] Outcome evaluated at 4 months. [2] Outcome evaluated at 6 months.

Furthermore, comparing these retrospective results with other trials evaluating the impact of scleroembolization practice alone at 6 months [7,27], the Resveratrol-based nutraceutical treatment intervention associated with surgical scleroembolization analyzed in this retrospective study clearly shows an interesting improvement in terms of sperm quality and recovery time of normal sperm parameters in just 4 months, so as to justify further investigation of this association to clinical practice (Table 5).

Table 5. Improvements (%) after scleroembolization at 4 and 6 months.

Outcome	%Incr (4 Months) Italiano et al. 2024	%Incr (6 Months) D'Andrea et al. 2019 [27]	%Incr (6 Months) Mancini et al. 2019 [7]	Δ (%)
Sperm Total Number	91%	479.2%	122.3%	−388.2 [27] −31.3 [7]
Sperm Total Motility	75%	-	6.8%	+68.2 [7]
Normal Morphology	64.6%	42.9%	59.6%	+21.7 [27] +5 [7]

Data are reported as the average increase (%) of the sperm total count, sperm total motility, and normal morphology. Patients' demographic data [7,27]: Age (years) = 35.0 (30.8–39); left varicocele grade II or III Dubin at physical examination and a continuous left-side SVR > 3 cm/s at scrotal CDUS, submitted to VR by retrograde internal spermatic vein embolization. D'Andrea et al. 2019 [27]. Age (years) = 16.6 (13–19); left varicocele grade II or III Dubin with Tanner stage 3–5. Mancini et al. 2019 [7].

4. Discussion

The impact of varicocele on sperm production and fertility is well known, and many mechanisms have been proposed to be involved in the pathophysiology. Inflammation, apoptosis, and oxidative stress all impact the seminal parameters and are all altered by varicocele. Many recent studies have shown that Resveratrol can impact the seminal parameters and affect fertility overall.

Indeed, in subfertile men, varicocele is associated with oxidative sperm damage and sperm DNA fragmentation, which are hypothesized to contribute to subfertility [28]. Treatment with Resveratrol is associated with decreased oxidative stress, inflammation, and sperm DNA fragmentation [19,20] and thus increases the likelihood of pregnancy and could be useful in improving the clinical parameters even in patients with more difficult recovery from surgery.

A recent pilot clinical study showed a significant increase in the number, concentration, and motility of spermatozoa among idiopathic infertile men [10]. Other studies have also shown biological benefits of Resveratrol in infertile women undergoing ICSI-IVF procedures [29], possibly by promoting mitochondrial biogenesis in granulosa cells [30].

Although the exact mechanism of Resveratrol remains to be understood in the spermatogenesis process, the possible anti-inflammatory and antiapoptotic effects could surely impact the seminal parameters. Noteworthy are the mitochondrial effects promoted by Resveratrol. It has been clearly demonstrated that the mitochondrial membrane potential and function is strictly related to sperm motility [28], and there is also evidence from other studies that Resveratrol affects the molecular mechanisms by which calcium signaling

impacts the functional outcomes in granulosa cell metabolism and mitochondrial biogenesis and that these mechanisms may impact follicle maturation [30]. Therefore, we can assume that Resveratrol may also similarly impact Sertoli cells, which contribute to normal spermatogenesis, mainly due to their influence on the nutrient supply, maintenance of cell junctions, and assist in gametic cell mitosis and meiosis.

However, only further preclinical studies will be able to highlight the complexities of sperm differentiation and how Sertoli cell modulation by Resveratrol may contribute to promoting healthy spermatogenesis and possibly also correct mitochondrial dysfunction and a decline in sperm motility during the spermatogenesis process.

All these considerations are interesting, because mitochondria also control many crucial functions of spermatozoa, providing the energy needed for their motility and ensuring a minimum concentration of reactive oxygen species, which, in the physiological range, contribute to sperm maturation, capacitation, and acrosome reaction. On the other hand, functional or structural dysfunction at the level of sperm mitochondria generates an overproduction of reactive oxygen with consequent oxidative stress and impaired energy production, leading to sperm DNA damage, decreased sperm motility, and worsening of the semen parameters and thus reduced male fertility. These aspects underline the pivotal role of mitochondria in male fertility [31].

The Resveratrol-based nutraceutical studied in this clinical trial also contained magnesium, vitamin D, vitamin B6, vitamin B12, and folic acid, nutraceutical substances that may have an impact on the semen parameters.

As an example, the literature has reported the positive effects of vitamin B12 on sperm quality in terms of improved sperm number and sperm motility and reduced sperm DNA damage [32]. Folic acid is also reported to significantly increase sperm density in patients with oligospermia or asthenospermia [33]. On the contrary, so far, no statistical correlations have been found between the amount of seminal plasma vitamin B6 and the seminal parameters [34]. Moreover, vitamin D is also directly implicated in ameliorating the semen quality and sperm motility, male reproductive potential, and testosterone levels [35,36]. Probably, this is due to the ability of vitamin D to promote the synthesis of ATP through the cAMP/PKA pathway [36]. However, to date, the role of vitamin D and other vitamins in male fertility still remains debated [34,37].

This present pilot retrospective trial shows an overall statistically significant spermiogram improvement in just 4 months and an increase in the spontaneous pregnancy rate after scleroembolization, clearly demonstrating the added benefit of a nutraceutical approach based on Resveratrol and vitamins in clinical practice.

The combined treatment led to a significant average increase in the total sperm number, total motility, and morphology that was not significant in the control patients. However, a strict comparison between the study group and control group could not be evaluated, considering that not all the demographic baseline data were homogeneous (Supplementary Table S1). It is interesting to note that, if you consider the sperm total number, starting from comparable baseline values (18.03 $\pm$ 10.59 in the study group vs. 16.45 $\pm$ 8.38 in the control group, p = 0.63), only a significant increase was found for the study group (T_0: 18.03 $\pm$ 10.59, T_4: 32.30 $\pm$ 20.60, p = 0.000011 vs. T_0: 16.45 $\pm$ 8.38, T_4: 19.50 $\pm$ 9.81 in the control group, p = 0.43) (Tables 2 and 3).

It is interesting to note that this combined approach allows restoring the semen parameters in a shorter time with respect to the average time reported as the normal practice (generally, more than 6 months). Furthermore, in support of this, one can consider the outcomes obtained in studies evaluating the impact of scleroembolization practice alone [7,27]. It is important to point out that the seminal parameter values derived from the work of Mancini [7] and D'Andrea [27] and used for the comparison were taken at 6 months from the groups with positive outcomes in scleroembolization practice (disappearance of left spermatic vein reflux (SVR)), which would therefore reasonably have had better restoration of the seminal parameters than the groups with a persistent SVR at the scrotal color-Doppler ultrasound (CDU) after varicocele repair. Therefore, these results clearly

show the interesting improvement of the seminal parameters at 4 months rather than those reported in the literature at 6 months [27] and, in some cases, even to a greater extent (Table 5). In particular, the average increase in the sperm parameters at 4 months from the present work was interestingly better with respect to the average increase in the sperm parameters in the works of Mancini [7] and D'Andrea [27] at 6 months in terms of the sperm total motility (75% increase vs. 6.8% increase; see Table 5) and normal morphology (64.6% increase vs. 42.9% and 59.6% increases; see Table 5). On the contrary, there is not a better result in terms of the average increase (%) of the sperm total number (91% increase vs. 479.2% and 122.3% increases; Table 5).

Moreover, in support of this, the results reported by the EAU Guidelines 2024 [3] regarding the surgical resolution of varicocele depicted significant improvements in terms of the pregnancy rates and total sperm count found between T0 and T12 [38] and also significative differences in sperm motility and normal sperm morphology between scleroembolized and untreated patients [39] at 6 or 12 months, showing how time is important for a complete recovery.

Considering these obtained results, this study may suggest that nutraceutical therapy based on Resveratrol and vitamins in combination with varicocele repair may have a positive effect on the sperm quality and recovery time of normal sperm parameters, with a beneficial effect on the couple's fertility.

This study, however, does have some limitations. The study is a pilot retrospective study, meaning it lacks blinding and randomization. A comparison with a small control group of 20 patients with Dubin II and III varicocele who did not receive any post-surgical nutraceutical treatment was evaluated; however, it was not possible to effectively compare all the variables, because not all baseline data were homogeneous between the two groups (Supplemental Table S1). Changes in the semen parameters were evaluated at 4 months after varicocele repair, while a second observation time might have been of interest in better evaluating the recovery of the parameters. Furthermore, a follow-up at 6 months after varicocele repair to obtain the pregnancy rate might be too short to properly assess the outcome and could be extended in a future work. Further prospective placebo-controlled studies could confirm the role of Resveratrol-based nutraceuticals in improving clinical practice in terms of timing and the extent of recovery of the seminal parameters in men with clinical varicocele submitted to varicocelectomy and can assess the true extent of this synergistic approach.

5. Conclusions

A Resveratrol-based nutraceutical therapy combined with surgical scleroembolization may lead to improved seminal parameters and may reduce the time needed to fully recover sperm function. The exact mechanisms are still poorly understood; perhaps, the improvement of oxygenation is due to surgical repair together with the impact of Resveratrol on cellular metabolism and could create a positive effect in restoring the physiological process of spermatogenesis. Altogether, these effects may contribute to reduce the time needed to fully recuperate sperm function, benefiting the fertility potential of couples.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jcm13102925/s1: Table S1: Control patient's demographic data at baseline.

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Institutional Review Board Statement: Not applicable. The study is considered a retrospective trial, and the National Code on Clinical Trials has declared that ethics approval is not necessary for real retrospective studies [40].

Informed Consent Statement: Patient consent was waived due to due the retrospective study design.

Data Availability Statement: Data supporting the reported results are available upon request. To request the data, contact the corresponding author of the article.

Conflicts of Interest: The authors declare that this research was conducted in the absence of any commercial or financial relationships. R.G.I. and G.C. are employees of S&R Farmaceutici S.p.A.

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Resveratrol-Based Multivitamin Supplement Increases Sperm Concentration and Motility in Idiopathic Male Infertility: A Pilot Clinical Study

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Abstract: Background. It is known that a multitude of factors may lead to male factor infertility, but still, in the majority of cases, the cause remains largely idiopathic, reflecting poor understanding of the basic process of spermatogenesis and the mechanisms involved. Resveratrol is a polyphenol compound that displays several cellular aspects mainly associated with SIRT1-pathway activation and promotion of mitochondrial enhancer activities. In several animal models, resveratrol has shown positive effects on mitochondria and membrane potential. This could explain effects on sperm concentration and motility. The aim of this study is to evaluate the effects on the semen parameters of GENANTE®, a multivitamin supplement containing 150 mg of resveratrol/day, in patients with idiopathic infertility. Methods. This was a prospective single center clinical study. Twenty patients took a multivitamin supplement based on 150 mg of resveratrol (GENANTE®), in the form of an oral tablet every 12 h, and were followed up at 1, 3, and 6 months after treatment. Pre- and post-treatment evaluation included history, clinical examination, semen analysis, hormonal determinations, and scrotal and prostatic ultrasound. Results. Our preliminary pilot study demonstrated that the multivitamin supplement based on resveratrol improves sperm motility (48.3% ± 13.8 vs. 59.0% ± 12.8, p = 0.0001) and concentration (22.6×10^6/mL ± 9.5 vs. 25.7×10^6/mL ± 8.1, p = 0.0001) after 3 and 6 months of treatment in men with idiopathic infertility. Conclusion. Our data suggest that targeting the metabolic and energetic pathways involved in spermatogenesis and mitochondrial activity could lead to potential effects and counteract subfertility/infertility in men through a mitochondria dynamics mechanism. Trial registration number: ClinicalTrials.gov registration identifier: NCT03864198, registered on 1 January 2019.

Keywords: resveratrol; male infertility;-mitochondrial activity

1. Introduction

Infertility is defined as the inability to achieve spontaneous pregnancy after at least one year of regular, unprotected sex [1]. Infertility affects 15–20% of couples [2]. A male factor is estimated to be present in approximately 50% of cases, with sole responsibility in 30% of cases and a co-contributing female factor in 20% of cases [3]. Male infertility may include the abnormal semen parameters (oligozoospermia, asthenozoospermia, teratozoospermia) or a combination of all three (oligo-astheno-teratozoospermia), or azoospermia [4]. The causes of male infertility can be divided into four

main areas (endocrine and systemic disorders, primary testicular defects in spermatogenesis, sperm transport disorders), including idiopathic infertility which affects up to 25% of patients [4]. Idiopathic male infertility is clinically diagnosed after excluding all other known causes of infertility.

Semen quality has often been used as an indirect measure of male infertility. This includes examination of sperm count, motility, and morphology. The majority, 80%, of altered parameters accounting for low sperm concentration are associated with a decrease in sperm motility (asthenozoospermia) and spermatozoa with normal morphology [5]. Whether there is a deterioration of semen quantity or quality is controversial [6,7]. However, there seems to be a clear trend toward a decline in sperm quality in our society [8]. There have been several explanations for this phenomenon. They include environmental stress, a modern lifestyle, infection, and/or chemicals that may alter the endocrine system. The result is a steady decline in male reproductive potential [9].

It is also known that in most infertile men who have abnormalities in sperm count, morphology, and/or motility, there is no identifiable cause [4]. Numerous nutritional [10–14] and medical interventions (hormonal therapies that modulate the hypothalamic–pituitary–testicular axis) have been used to treat male idiopathic infertility [2]. However, the management of these patients remains challenging. This is primarily due to the large numbers of various products and the conflicting evidence from individual trials. Most of the studies on infertility treatment, both in vitro and in vivo, have focused on oxidative stress mechanisms [15–17]. The oxidative stress mechanism could cause: lipid peroxidation, with alteration of membrane fluidity and permeability, which results in a decrease of sperm motility and in a reduction of sperm interaction with the oocyte; protein modification which causes a reduction of ATP production; or sperm DNA fragmentation [16].

In particular, the focus has been on increasing seminal antioxidant capacity, reducing the production of reactive oxygen species (ROS), stabilizing sperm chromatin (through zinc-based molecules), and inducing sperm capacitation (functional maturation of the spermatozoa). By contrast, few studies have centered on other mechanisms involved in metabolism, mitochondrial energy, and metabolism/mitochondrial function, which has recently emerged as one of the important factors in infertility physiopathology [18,19].

Resveratrol, trans-3,5,4′-trihydroxystilbene, is a polyphenol compound present in grapes, peanuts, berries, and wine [13]. It is a phytoalexin whose biological function is to protect the plant in case of parasitic attack or environmental stress [14]. Scientific reports have identified a wide variety of characteristics of this molecule. This includes anti-inflammatory, cardioprotective, anticancer, antimicrobial, antiaging, and antioxidant effects [20]. Resveratrol is also the most potent natural compound that activates sirtuin 1 (SIRT 1), the most-conserved mammalian NAD+-dependent protein, and a member of the family of sirtuins, which may account for its many metabolic benefits in humans [20].

Recent studies in animal models have demonstrated that resveratrol has a positive effect on the hypothalamic–pituitary–gonad axis, as well as blood testosterone levels, sperm production, and sperm motility [21,22]. Furthermore, resveratrol may decrease germ cell apoptosis [18,23]. An animal study with resveratrol and lycopene in post-thaw bull sperm demonstrated that resveratrol offered high mitochondrial activity, sperm motility, and DNA integrity [24]. It improved DNA integrity and sperm parameters in streptozotocin-nicotinamide-induced type 2 diabetic rats [25]. In vitro treatment with 15μM/mL of resveratrol on human sperm revealed that it has the capability to counteract the detrimental effects of benzo-α-pyrene exposure on sperm motility, abnormal chromatin compactness, lipid peroxidation, and mitochondrial superoxide [26]. Resveratrol can protect the quality of the mitochondria and increase its membrane potential [27], an effect that possibly accounts for the positive effect on sperm motility and explains the improvement of total and progressive sperm motility.

Despite these insights, the effect of resveratrol supplementation on male infertility has not yet been explored.

The aim of this study was to evaluate the effects of a nutraceutical based on resveratrol, (GENANTE®, a twice-a-day multivitamin supplement containing 150 mg of resveratrol, vitamin D,

B6, B12, and folic acid) on the semen parameters of patients with idiopathic infertility. The primary outcome was to evaluate the semen parameters before and after 1, 3, and 6 months of treatment.

2. Material and Methods

This was a prospective single center study. The project was accepted by the local ethics committee and registered on clinicaltrials.gov (NCT03864198). We included idiopathic infertile male patients. The inclusion criteria were as follows: age 18–50 years and patients with oligozoospermia (<5 million spermatozoa/mL) and/or with asthenozoospermia (<32% progressive motile spermatozoa) e/o in accordance with WHO criteria [28]. The following patients were excluded: patients with azoospermia; patients who smoked and/or used drugs, or had taken drugs with proven fertility toxicity; patients with a history of consumption of alcohol; those who were exposed to any environmental or occupational toxic substances, including radiation, intensive cell-phone use or heat (patients who claimed to have a mobile phone in their front pocket for at least 5 h, for at least 10 min/h); patients who had epididymitis, epididymo-orchitis or orchitis secondary to mumps, bacterial infections, or sexually transmitted diseases; patients with a history of cryptorchidism, previous testicular torsion, genitourinary anomalies, alterations of the epididymis or deferens, and/or inguinal surgery; patients with hormonal alterations. The reason for exclusion was the causal relationship of these conditions with the deterioration of fertility [29–31].

The pretreatment evaluation included a patient history; clinical examination; semen analysis; hormonal determination (follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol, prolactin, and 25-OH-Vitamin D3); a scrotal ultrasound to exclude signs of obstruction (e.g., dilatation of rete testis, enlarged epididymis with cystic lesions, or absent vas deferens), signs of testicular dysgenesis (e.g., non-homogeneous testicular architecture and microcalcifications), or testis tumors; and a prostatic transrectal ultrasound to exclude distal obstruction and any male accessory gland infection [32,33].

Hormonal assessments were performed in the same laboratory, and their evaluation was based on reference ranges for normal men provided by the laboratory measuring the samples.

In our laboratory, normal ranges were: prolactin 3.46–19.40 ng/mL; FSH 0.95–11.95 mUI/mL, LH 1.14–8.75 mUI/mL; total testosterone 10–20 years: 18.5–48.3 pg /mL, 20–30 years: 19.5–51.7 pg/mL, 30–50 years: 16.1–47.9 pg/mL, >50 years 12.1–39.6 pg/mL; estradiol 11–44 pg/mL; 25-OH-Vitamin D3 < 20 ng/mL deficiency, 20–29 ng/mL insufficient, 30–100 ng/mL sufficient. All assessments were certified (certified quality system UNI EN ISO 9001:2015).

Laboratory testing of testosterone to determine diurnal variation was carried out with two morning samples (7.00 a.m. and 11.00 a.m.). Prolactin levels are influenced by several factors, such as pick-up time (the prolactin secretion has a circadian rhythm, with high levels in the night and low during the day). However, since it was not possible to do so during sleep, dosage was carried out during the day, with three samples taken at intervals of 10–30 min. Estradiol, FSH, and LH were assayed by one sample.

Scrotal and transrectal ultrasounds were performed by the same urologist.

Patients were prescribed a multivitamin supplement (trademark GENANTE®, S&R Farmaceutici S.p.A. Bastia Italy). It consisted of REVIFAST® (160 mg), trans-resveratrol (102 mg), Vitamin B6 (1.4 mg), Vitamin B 12 (2.5 mg), Vitamin D (25 mcg), and Extrafolate S® (400 mcg). They received an oral tablet every 12 h, for a total of 2 tablets a day, for a daily consumption of 150 mg of resveratrol.

REVIFAST® is the trade name of a new ingredient based on resveratrol that is supported by a magnesium hydroxide matrix at the concentration of 30% w/w [34]. Resveratrol is known to have low solubility in water and good membrane permeability and accordingly is a class 2 molecule by pharmaceutical classification, and therefore, it is poorly bioavailable [34]. Furthermore, it is known to have a fast metabolism that converts it to glucuronide and sulfate compounds.

Extrafolate S® is the biologically active form of folic acid. This allows it to bypass any polymorphisms of the tetrahydrofolate methylene (MTHFR) gene reductases responsible for reduced enzyme activity of MTHFR.

All patients were followed at 1, 3, and 6 months after treatment with Genante© using the same pretreatment flow chart. Scrotal and transrectal ultrasounds were performed during follow up to rule out de novo pathologies.

All patients signed an informed consent form explaining the nature of the study and the possibility of treatment failure.

Statistical Analysis

With the enrolment of 20 patients, $p = 0.05$, and the use of the $\chi 2$ test, the study was estimated to have an 80% power rejection of the null hypothesis that Genante® does not change the seminal parameters in infertile patients. The power of the study was calculated using PS Power and Sample Size ver. 3.0, 2009. Continuous variables were presented as median values, and categoric data were presented as absolute or relative frequencies. Statistical analysis was performed using the Wilcoxon Signed Rank test to compare the variables, and the $\chi 2$ test and McNemar test for categorical data. All calculations were performed using IBM-SPSS® version 22.0 (IBM Corp., Armonk, New York, NY, USA, 2013). A two-sided p-value < 0.05 was considered significant.

3. Results

Between January 2019 and June 2019, 20 patients, with idiopathic infertility according to WHO criteria, underwent treatment with Genante® in our tertiary urological center. The demographic and clinical characteristics of the included patients are shown in. Table 1.

Table 1. The demographic and clinical characteristics of patients.

Patients	20
Age (mean ± SD)	30.9 ± 3.28
BMI (mean ± SD)	27.9 ± 1.4
Married *n* (%)	10 (50)
Erectile Dysfunction *n* (%)	0 (0)
Male hyopogonadism *n* (%)	0 (0)
Oligozoospermia *n* (%)	0 (0)
Asthenozoospermia *n* (%)	6 (30)
Teratozoospermia *n* (%)	7 (35)
Oligoasthenozoospermia *n* (%)	7 (95)
Oligoteratozoospermia *n* (%)	0 (0)
Asthenoteratozoospermia *n* (%)	0 (0)
Oligosthenoteratozoospermia *n* (%)	0 (0)
Normal scrotal ultrasound	20 (100)
Normal prostatic transrectal ultrasound	20 (100)
FSH mlU/mL (mean ± SD)	4.60 ± 1.3
LH mlU/mL (mean ± SD)	3.87 ± 1.8
Total Testosterone nmol/L (mean ± SD)	14.89 ± 0.3
Estradiol pg/mL (mean ± SD)	25.2 ± 2.1
Prolactin ng/mL (mean ± SD)	11.17 ± 1.9
25-OH-Vitamin D3 ng/mL (mean ± SD)	55.8 ± 2.8

FSH: follicle-stimulating hormone. LH: luteinizing hormone.

Half of the patients were married; the other half had stable relationships, but they were not married. The most frequent sperm abnormality among the included patients was oligoasthenozoospermia (95%). At pretreatment, all patients had normal transrectal prostatic ultrasounds and scrotal ultrasounds.

After six months of treatment, the laboratory assessment showed a statistically significant improvement in total sperm count (41.5×10^6/ejaculate ± 22.1 vs. 48.2×10^6/ejaculate ± 22.4, $p = 0.002$),

sperm concentration (22.6 × 10^6/mL ± 9.5 vs. 25.7$10^6$/mL ± 8.1, $p = 0.0001$), total motility (48.3 ± % ± 13.8 vs. 59.0 ± % ± 12.8, $p = 0.0001$), and progressive motility (20% vs. 90%, $p = 0.0001$) (Table 2 and Figure 1).

The improvement of all the parameters was recorded previously at 1 and 3 months, with a progressive increase over time (Table 2). Sperm morphology, volume, and PH were not changed after treatment. The hormonal determinations were rather stable during follow-up (Table 3). The scrotal and transrectal prostate ultrasounds continued to be in the normal range.

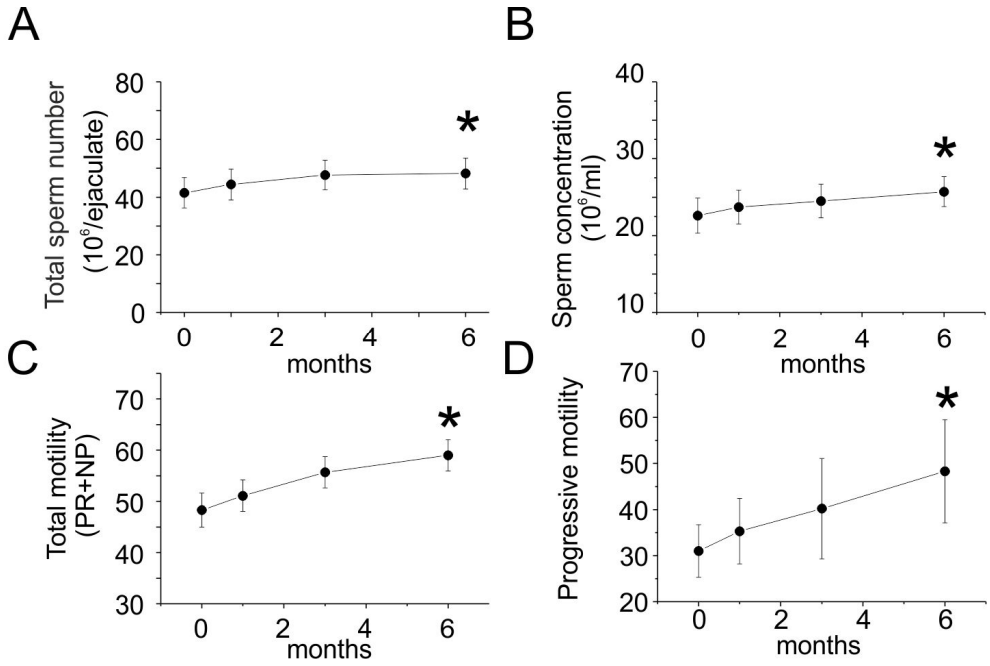

Figure 1. The laboratory assessment after six months of treatment. Data presented as ± SD. Significant differences between control and treated cells are denoted as * ($p \leq 0.05$). (**A**): change in the total number of spermatozoa in six months. (**B**): change in the sperm concentration in six months. (**C**): change in the total motility (PR+NP) in six months. (**D**): Change in progressive motility in six months.

Table 2. Semen parameters at baseline 1, 3, and 6 months after recruitment.

Parameters	Baseline	1 Month	3 Months	6 Months	*p*-Value
Normal Viscosity *n* (%)	20 (100)	20 (100)	20(100)	20 (100)	nd
Complete Fludification *n* (%)	11(100)	11(100)	11(100)	11(100)	nd
PH (mean ± SD)	8.1 ± 0.1	8.0 ± 0.3	8.0 ± 0.2	8.0 ± 0.3	0.219
Semen volume (mL, mean ± SD)	3.6 ± 0.7	3.61 ± 0.6	3.61 ± 0.5	3.7 ± 0.5	0.525
Total sperm number (10^6/ejaculate, mean ± SD)	41.5 ± 22.1	44.4 ± 22.4	47.7 ± 21.4	48.2 ± 22.4	0.002 *
Sperm concentration (10^6/mL, mean ± SD)	22.6 ± 9.5	23.7 ±9.2	24.5 ± 9.1	25.7 ± 8.1	0.0001 *
Total motility (PR + NP, % mean ± SD)	48.3 ± 13.8	51.1 ± 12.8	55.7 ± 12.7	59.0 ± 12.8	0.0001 *
Progressive motility (PR > 32% mean ± SD)	31 ± 5.7	35.3 ± 7.1	40.2 ± 10.9	48.3 ± 11.2	0.0001 *
Sperm morphology (normal forms%)	14 (66.7)	14 (66.7)	14 (66.7)	14 (66.7)	nd

PR: progressive motility. NP: non-progressive motility. nd: not determined * ($p \leq 0.05$).

Table 3. Hormonal evaluation at baseline 1, 3, and 6 months after recruitment.

Parameters	Baseline	1 Month	3 Months	6 Months	p Value
FSH mlU/mL (mean ± SD)	4.60 ± 1.3	4.62 ± 1.2	4.61 ± 1.5	4.61 ± 1.7	0.9
LH mlU/mL (mean ± SD)	3.87 ± 1.8	3.85 ± 1.3	3.87 ± 1.4	3.88 ± 1.4	0.87
Total Testosterone nmol/L (mean ± SD)	14.89 ± 0.3	14.84 ± 0.7	14.82 ± 0.2	14.87 ± 0.2	0.9
Estradiol pg/mL (mean ± SD)	25.2 ± 2.1	25.8 ± 2.4	25.5 ± 2.3	25.6 ± 2.0	0.86
Prolactin ng/mL (mean ± SD)	11.17 ± 1.9	11.13 ± 1.5	11.15 ± 1.3	11.19 ± 1.2	0.9
25-OH-Vitamin D3 ng/mL (mean ± SD)	55.8 ± 2.8	55.1 ± 2.2	55.7 ± 2.4	55.4 ± 2.1	0.9

FSH: follicle-stimulating hormone. LH: luteinizing hormone.

4. Discussion

It is known that a multitude of factors may lead to male factor infertility; however, in the majority of cases, the cause remains largely idiopathic, which reflects a poor understanding of the basic process of spermatogenesis and the mechanisms involved [19].

Sperm density may be influenced more by many nutraceuticals or micronutrients, such as vitamin D, B and folic acid, while limited nutrients influence sperm motility. Folic acid is known to increase sperm density significantly following three months of folic acid supplementation to patients with oligospermia or asthenospermia [35]. By contrast, no statistical correlations were found between seminal plasma vitamin B6 level and sperm motility, sperm count, or semen volume [36]. Folate and B12 are not correlated with any semen parameters [37] but are known to modulate homocysteine. Vitamin D demonstrates a direct and positive relationship between serum vitamin D level and overall semen quality, male reproductive potential, and testosterone levels [38] and may enhance sperm motility by promoting the synthesis of ATP through the cAMP/PKA pathway [39]. However, in the literature, controversial data exist regarding vitamin D status and reproductive parameters [40], and thus, the role of vitamin D in male fertility is still debated.

Our study shows that a resveratrol-based multivitamin treatment increases the concentration of sperm cells and motility, which suggests an improvement in both the spermatogenesis process and fertilization potential. The process of spermatogenesis comprises the differentiation of the primordial germ cells into spermatogonia, followed by the production of primary and secondary spermatocytes, spermatids, and ultimately highly specialized mature spermatozoa [41]. Sertoli cells (SCs) play a key role in spermatogenesis by providing the essential physical support for developing germ cells and ensuring that they have the appropriate nutrients, energy sources, hormones, and growth factors.

In fact, spermatogenesis is highly dependent on energy metabolism [42] and glycolytic metabolism, as the lactate produced by the Sertoli cells is the major substrate of germ cells [43]. The mitochondria of the isolated germ cells produce ATP potentially, at close to a maximal rate. Spermatogenesis, therefore, may be extremely sensitive to compounds which interfere with mitochondrial energy metabolism and respiratory control. Any alteration in the regulation of these cells' metabolic behavior may compromise the normal development of spermatogenesis and, consequently, male fertility [42,43]. It has been proposed that mitochondria also play a role in this degenerative process of the sperm, thereby assuring that good quality meiotic products enter the process of spermatogenesis to yield quality mature sperm [19]. Interestingly, resveratrol was demonstrated to increase the mitochondrial number (mitogenesis) and activity (ATP concentration) in several cell types, such as muscle cells [20] and granulosa cells [44]. Resveratrol improves mitochondrial function by activating sirtuin 1 (SIRT1) [20]. SIRT 1 is related to multiple age-associated diseases due to its capacity to deacetylate histones and non-histone proteins, such as tumor protein p53 (p53), kB-gene binding nuclear factor (NF-κB), heat shock factor 1 (HSF1), forkhead box transcription factor, class O (FOXOs), and peroxisome proliferator-activated receptor γ (PPARγ)

coactivator-1 (PGC-1). Thus, it can regulate the cell's biology, metabolism, and fate at various levels [45]. Therefore, sirtuins play an important role in a broad spectrum of biological processes. Their regulation of glycolytic metabolism and mitochondrial energy metabolism–respiratory control not only increases their physiological relevance to the testicular environment [46]; however, it also suggests that these metabolisms control sperm functionality and thus male reproductive health. Further studies are required to conclusively demonstrate if this effect on males occurs in male gamete cells during resveratrol treatment and if it is dependent on the SIRT-1 pathway.

Mitochondrial activity is also critical for mature sperm cells as it is correlated with sperm motility, an important factor for the penetration of the cumulus cells and zona pellucida of the oocyte [23]. In mature sperm, mitochondria cover the axosome and the associated dense fibers of the midpiece, via oxidative phosphorylation (OXPHOS), which increases the production of adenosine triphosphate (ATP) [18]. The inner mitochondrial membrane includes several complexes (electron transfer chain, ETC), which transport electrons derived from the oxidation of dihydroflavine–adenine dinucleotide (FADH2) and the nicotinamide adenine dinucleotide (NADH). In this process, an osmotic proton gradient is generated across the inner mitochondrial membrane and is subsequently used by the ATP synthase to phosphorylate adenosine diphosphate (ADP) to ATP. OXPHOS-derived ATP seems to be important for sperm motility [18]. The expression of several sperm mitochondrial proteins, including ETC complexes [47], may be altered in asthenozoospermic patients [23,48]. Many different ETC inhibitors have been shown to negatively affect sperm motility [49], both in humans [49,50] and in engineered mice [51]. Interestingly, there is a strong correlation with inner mitochondrial membrane potential (DP) and spermatic motility. In this context, the increase of motility observed in our study due to resveratrol treatment may be associated with this incremented mitochondrial membrane potential and metabolic activity. Mitochondria, in the mid-piece of mature mammalian spermatozoon, are fundamental for the creation of energy which is useful for sperm movement [19], and mtDNA genetic defects may compromise sperm physiology, and, in particular, motility [19]. Multiple mtDNA rearrangements are associated with decreased sperm motility [52]. In addition, the reduction of energy production may induce meiotic arrest during spermatogenesis [19].

It is unlikely that this is an increase in the expected number of mitochondria as the number of mitochondria is highly dependent on the neck volume of sperm cells. The beneficial effect of resveratrol agrees with the inverse correlation of mtDNA; in fact, mtDNA would be advantageous to developing spermatozoa [19], but not in mature sperm cells. Oligozoospermic and asthenozoospermic men have sperm containing significantly elevated levels of mtDNA [53], prompting the hypothesis of an optimal threshold for spermatozoa. Since the energy metabolism is important in both spermatogenesis and oxidative phosphorylation, it has been suggested as a determinant of sperm motility and functionality.

We are aware that this study may have some limitations. One is related to its small sample size and study design as a prospective clinical study. The second is the lack of evaluation of the impact of redox status in the effects observed since mitochondrial ETC promotes the production of ROS [18]. Balanced ROS levels are required for sperm motility, capacitation, the acrosome reaction, hyperactivation, and fertilization ability [27], so we can assume that ROS levels are inside of physiological range; however, further studies will be able to address the specific role of ROS in resveratrol's effects in promoting a better spermatic performance. The strengths of our study include the use of a supplement with a highly bioavailable form of resveratrol (REVIFAST™) with an increased pharmacokinetic profile [34]. It can bridge the gap between the interesting in vitro effects that are otherwise not possible in vivo.

5. Conclusions

In conclusion, GENANTE® improves the concentration and motility of sperm in idiopathic male infertility. This study also confirms that, taken together, the possibility of targeting the metabolic and energetic mechanisms involved in spermatogenesis and sperm motility with a promising molecule such as resveratrol could provide clinical benefits. However, a deeper understanding of the specific mechanisms involved is essential and further studies are needed to confirm our hypothesis.

Abbreviations

ADP	phosphorylate adenosine diphosphate
ATP	production of adenosine triphosphate
DP	mitochondrial membrane potential
ETC	electron transfer chain
FADH2	dihydroflavine-adenine dinucleotide
FOXOs	forkhead box transcription factor, class O
FSH	follicle-stimulating hormone
HSF1	heat shock factor 1
LH	luteinizing hormone
MTHFR	polymorphisms of the tetrahydrofolate methylene
NADH	nicotinamide adenine dinucleotide
NF-kB	kB gene binding nuclear factor
OXPHOS	oxidative phosphorylation
PPARγ	peroxisome proliferator-activated receptor γ
SIRT1	sirtuin 1

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