

Predictive parameters of Semen Outcome Improvement After Varicocele Treatment by Scleroembolization: a Retrospective Study

Luca De Toni^{1*}, Giordana Ferraioli^{1*}, Matteo Todisco², Sara Congiu¹, Pierfrancesco Palego¹, Andrea Graziani¹, Nicola. Caretta¹, Giuseppe Grande¹, Giorgio De Conti², Massimo Iafrate³, Fabrizio Dal Moro³, Alberto Ferlin¹, Andrea Garolla^{1#}

Short Title: Predictors of semen outcome in varicocele treatment

* The two authors equally contributed to the study

Affiliations

¹ Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padova, Italy

² Unit of Radiology, Padova University-Hospital, Padova, Italy

³ Unit of Urology, Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy

#Correspondence: Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padova, Italy, Via Giustiniani 2, 35128 Padova, Italy (telephone: +39 049 8218518; e-mail: andrea.garolla@unipd.com).

Data Availability Statement: The data that support the findings of this study are available from the Padova University-Hospital but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Padova University-Hospital. For any information, contact luca.detoni@unipd.it

Conflict of Interest: The authors have no conflicts of interest to disclose

Funding: None

Key Words: Follicle stimulating hormone; total motile sperm count; dynamic ultrasound with contrast agent; mean transit time,

Abstract

Purpose Varicocele is a recognized male factor of infertility. Surgical/microsurgical correction represents a therapeutical option to improve fertility outcomes but predictive parameters for the identification who can actually benefit from varicocele correction are underinvestigated. Here we aimed to identify baseline predictors of semen outcome improvement after varicocele treatment by scleroembolization approach.

Methods 85 patients receiving varicocele treatment by antegrade scleroembolization (ASE, N=42) or retrograde scleroembolization (RSE, N=43) were retrospectively recruited. Basal and 6-months follow-up evaluation of semen, hormonal and ultrasound parameters (US) were performed to address the respective effect of varicocele treatment. In addition, basal parameters were assessed as clinical outcome predictors.

Results Varicocele grade reduction was observed in more than 90% of patients ($P<0.001$). Compared to basal, significant increase of sperm concentration ($10.0\pm9.2\times10^6\text{cells/mL}$ vs $23.4\pm26.9\times10^6\text{cells/mL}$; $P<0.001$), total sperm count (TSC $39.0\pm54.5\times10^6\text{cells}$ vs $70.3\pm90.6\times10^6\text{cells}$, $P<0.001$) and total motile sperm count (TMS, $9.8\pm12.8\times10^6\text{cells}$ vs $36.7\pm58.1\times10^6\text{cells}$, $P<0.001$), was observed, with no differences between RSE or ASE. All US parameters were also improved (all $P<0.001$). Logistic regression analysis of basal semen and ultrasound parameters showed that basal sperm motility and left testis-mean transit time (L-MTT) were associated with TSC and TMS doubling at follow-up. However, Receiver Operating Characteristic curve analysis showed that only basal L-MTT basal sperm was consistently associated with TSC and TMS doubling at follow-up (respectively: AUC = 0.834, CI: 0.747-0.921 and AUC = 0.805, CI: 0.708-0.901; both $P<0.001$).

Conclusion Baseline sperm count and US parameters represent useful clinical descriptors to address those subjects eligible for varicocele correction.

74 **Introduction**

75 Varicocele is defined as an abnormal venous dilation within the pampiniform plexus of testis,
76 mostly associated with venous reflux [1]. With a typical onset during puberty, varicocele is a
77 common clinical condition in andrological practice, with an estimated prevalence of 15% within
78 the general population. Since the prevalence of varicocele rises to 35-80% in infertile patients, it
79 results as one of the most common reversible male factor of infertility (MFI) [2, 3]. However,
80 current hypotheses suggest varicocele to be actually responsible for a lower proportion of MFI
81 [4]. In approximately 90% of the cases varicocele is left-sided whilst, in the remaining 10%, is
82 bilateral with a higher involvement of the left side [2]. The diagnosis of varicocele associates
83 with major clinical outcomes such as left testicular hypotrophy, found in 20-35% of patients,
84 altered testicular-endocrine function and qualitative/quantitative impairment of semen
85 parameters, resulting in an overall reduction of fertility potential after the exclusion of other
86 major causes of MFI [5, 6]. Different pathophysiological mechanisms have been invoked to
87 explain the varicocele-associated testicular damage, from tissue hypoxia and oxidative stress to
88 the increased scrotal temperature associated with reduced heat dissipation [7–9]. Accordingly, a
89 recent study from our group highlighted a major role of the impairment of left testis
90 microcirculation in varicocele-related issues [10]. In particular, using a contrast-enhanced
91 ultrasound (US) approach, a significant correlation was found between left testis perfusion time,
92 evaluated by the mean transit time (MTT) US parameter, and semen parameters. Importantly, an
93 MTT value exceeding 36 seconds was proven to be an independent predictor of venous
94 intratesticular stasis and oligozoospermia [10].

95 Varicocele correction by either surgical/microsurgical or endovascular approaches represents a
96 therapeutical option to improve the fertility outcomes associated with this clinical condition.
97 However, considering the different degrees of invasiveness of surgical approaches and the
98 controversial results about the inconsistent improvement observed in testis function, a gold
99 standard strategy is not yet defined. Current guidelines from the American Urological
100 Association (AUA) and the European Urological Association (EAU) do not identify threshold
101 values, within available semen and hormonal parameters, according to which referring for
102 varicocele correction by microsurgical/endovascular treatment [11, 12]. In this frame, the
103 possible predictive role of the novel vascular characterization of the testis by US in current basal
104 assessment of varicocele, in addition to standard semen and hormonal parameters, is still poorly
105 investigated. Furthermore, the clinical benefit associated with varicocele correction in patients
106 with clear signs of primary testis disease, such as high serum follicle stimulating hormone
107 (FSH), azoospermia or severe oligozoospermia, is currently debated [13]. Indeed, available data
108 showed no major beneficial effect on azoospermia, cryptozoospermia or complete isolated
109 severe asthenozoospermia upon varicocele correction, being largely related to other etiological
110 factors such as genetics [14]. On these bases, a thorough clinical evaluation, including complete

anamnesis, medical/biochemical/ultrasound evaluation and complete semen analysis, remains a key step to address the characterization of MFI in patients [15].

In this study we aimed to identify reliable predictors of semen outcome, by the evaluation of a group of patients affected by varicocele, undergone to scleroembolization and characterized for semen, hormonal and ultrasound parameters.

Methods

Study design and patients

This was a retrospective observational study approved by the Territorial Ethics Committee Central - Eastern Veneto Area (study code# AOP3137). The study complied with the guidelines of the Helsinki Declaration [16]. All patients provided a signed informed consent for the retrospective study participation and were recruited at the Unit of Andrology and Reproductive Medicine of the University Hospital of Padova (Italy).

The analysis involved patients aged 18-55 years attending the outpatient Unit in the period January 2009-August 2025. Inclusion criteria were: diagnosis of varicocele associated with primary infertility and total sperm count (TSC) $\leq 40 \times 10^6$ cells/ejaculate according to WHO criteria [17] and adherence to the follow-up visit scheduled 6 months after treatment as per standard clinical management of varicocele. Exclusion criteria were: age ≤ 18 years or ≥ 55 years, other known causes of infertility, including with positive microbiological culture and/or semen detection of human papillomavirus (HPV), positive screening for human immunodeficiency virus (HIV), hepatitis C (HCV) or B (HBV) viruses, concurrent treatment with sex steroids or gonadotropins, diagnosis of severe neoplasms or psychiatric disorders, severe secondary pathologies that may interfere with the study results.

Patients were eligible for varicocele treatment according to current urological guidelines [18, 19]. The unbiased and virtually-random assignment of patients to either Tauber's anterograde scleroembolization (ASE), performed at the Unit of Urology, University-Hospital of Padova (Padova, Italy). or retrograde scleroembolization (RSE), performed at the Unit of Radiology, University-Hospital of Padova (Padova, Italy), was addressed per the earliest convenience based on waiting lists as standard clinical practice [20, 21]. All patients were evaluated at basal, before varicocele treatment, and at six months from intervention. Only patients in which procedural ASE or RSE was successfully accomplished were considered for the analysis. Patients in which procedural or anatomical factors unabled scleroembolization procedures, were excluded. All patients provided a peripheral blood sample, obtained by 8:00 am on a fasting state, for the evaluation of serum FSH, LH, total testosterone (TT), estradiol (E2) and prolactin (PRL) by electrochemiluminescence immunoassay methods (Cobas®; Roche Diagnostics, Mannheim, Germany). Semen analyses, performed according to the WHO laboratory manual for

examination and processing of human semen – editions from fourth to sixth, included: sperm concentration, total sperm count (TSC), motility (further distinguished in type a, progressive, type a+b, total progressive and non-progressive and type c, non-motile), viability, typical forms. In order to provide the harmonic evaluation of semen outcomes across the whole range of retrospective enrollment (January 2009-August 2025), semen parameters were considered only as raw continuous variables and eventually clustered into unbiased categorical variables as quartiles as described below. Total motile sperm (TMS) count TMS was a derived parameter obtained from the product of TSC $\times$ type a+b motility. All analyses were performed according to standard procedures at the Unit of Laboratory Medicine of the University Hospital of Padova. Semen evaluation was replicated two times both at basal and at follow-up and the mean value of replicates was considered for the analysis.

Sperm DNA fragmentation, chromatin integrity and aneuploidies assessment were performed by respectively: terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) test, acridine orange test and fluorescence in situ hybridization (FISH) tests as previously described [22].

Medical and pharmacological history of patients, including lifestyle habits, were extracted from medical records. Specifically, data included: dietary and smoking habits, alcohol consumption, physical activity, sexual habits, andrological evidence, such as trauma, infections and testicular surgeries, presence of rheumatological, renal, endocrinological, gastroenterological, neoplastic, psychiatric disorders, family history of testicular disorders and infertility and regular use of medications that may influence testicular function, such as sex steroids or gonadotropins. The andrological examination aimed to explore the structural and morphological characteristics of the penis, testicles, epididymis, vas deferens, spermatic cord structures, prostate and the assessment of secondary sexual characteristics. Particular importance was paid for the assessment of testicular volume by ultrasound analysis as detailed below. The physical examination was conducted in both supine and standing positions, both at rest and during a Valsalva maneuver. Patients missing at least one of clinical data considered were excluded from the analysis.

Scrotal doppler ultrasound and testicular contrast harmonic imaging

Patients were evaluated at dynamic ultrasound with contrast agent (US, Philips Epiq Elite, Milan, Italy) by the same trained medical staff (P.P. and N.C.) at all time points. US was performed with the patient lying in the supine position and the penis lifted towards the abdomen. A high-resolution linear Doppler probe (5-18 MHz) was used to collect transverse and sagittal images of both testes, in order to define volume, parenchymal echotexture, and perfusion status. Images were acquired and interpreted only by trained and experienced medical personnel (P.P. and N.C.). The US evaluation was performed according to most recent recommendations on scrotal

ultrasound [23]. Varicocele was categorized into a five degrees scale according to the recommendations or the Penile Imaging Working Group of the European Society of Urogenital Radiology (ESUR-SPIWG) and the European Academy of Andrology [24] (<http://www.andrologyacademy.net/studies>).

Testicular contrast harmonic imaging was performed by antecubital vein injection of 2.5 ml of a contrast agent composed of microbubbles of sulfur hexafluoride, stabilized with phospholipids, immediately followed by a rapid injection of 5 ml of 0.9% sodium chloride solution. A 3-minute video clip at 15 frames per second was recorded starting 20 seconds after the contrast agent injection. Video clips were automatically analyzed using the Qontrast[®] software (Bracco, Milano, Italy) as previously described [25]. The software processed the intensity-time curves in an operator-unbiased manner and calculated the time of arrival of the contrast agent in the arterial circulation (wash-in), the TTP, the time of arrival of the contrast agent in the venular circulation (wash-out), and MTT. The procedure was repeated for the contralateral testicle after a 10-minute interval to allow for complete elimination of the contrast agent.

Statistical analysis

Data were collected in a pseudo-anonymized dataset on a computer device and all statistical analyses were performed using SPSS software version 23.0 (SPSS Inc, Chicago, USA). The results were expressed as means $\pm$ standard deviation (SD). The prevalence of semen, hormonal, and ultrasound alterations was calculated in percentage and the Chi-Square test was used to assess prevalence differences through contingency tables. The Kolmogorov–Smirnov test was used to verify normal distribution of continuous variables. Comparison of mean values of continuous variables between two groups was performed by Student's *t*-test. Comparison between two or more groups showing non-normal distribution of continuous variables was performed by non-parametric Kruskal-Wallis test. Differences in the mean value of parameters between more than two groups was performed with the analysis of variance (ANOVA) test with Bonferroni's post-hoc test for pairwise comparisons between subgroups. Multivariate analysis with Bonferroni's post-hoc correction was used to evaluate seminal, hormonal, and ultrasound parameters upon patients' stratification. The association between a set of continuous with TSC or total motile sperm (TMS) doubling from basal as dichotomous outcome, was evaluated by logistic regression analysis, and the respective odds ratios (ORs) with 95% confidence intervals (CIs) was calculated. Receiver operating characteristic (ROC) curve was calculated by plotting sensitivity values on the y axis and 1-sensitivity values on the x axis. The area under the curve (AUC) was determined and evaluated by Sweets classification and the cutoff values for each considered variable have been calculated with Youden's *S* statistics. The significance level of *p* was set to 0.05.

Results

The retrospective analysis between January 2009-August 2025 allowed the recruitment of 85 patients with diagnosis of varicocele, 42 of which underwent to anterograde scleroembolization (ASE) according to Tauber's method whilst 43 underwent to retrograde scleroembolization (RSE). Clinical and demographic data of patients at basal, and further distinguished for scleroembolization approach, are reported in Table 1. The comparison of demographic, seminal, hormonal and US parameters showed no significant differences between RSE and ASE patients. Major differences among the two groups of patients were observed in regard of the varicocele grading according to ESUR-SPIWG classification, since grade III was more represented in RSE patients, whilst grade V was more represented in ASE patients (respectively: grade III 18/43 RSE vs 8/42 ASE and grade V 7/42 ASE vs 3/42 RES; $P=0.034$ at χ^2 test). Finally, we found a significant difference between left and right testis volume, being the left smaller respectively: left 11.8 ± 3.2 mL, right 14.5 ± 4.0 mL, $P<0.001$).

Basal to follow-up evaluation of patients

Results of the basal vs follow-up comparison of demographic, seminal, hormonal and US parameters of the whole study group is reported in Table 2. Independently from the scleroembolization approach, data from the overall study cohort showed that varicocele correction was associated with significant reduction of the grade of varicocele compared to basal ($P<0.001$), with more than 90% of patients displaying a degree equal or lower than one at follow-up evaluation. In addition, the comparison of hormonal and semen parameters at follow-up vs basal showed a significant reduction of FSH levels together with the increase of semen volume, sperm concentration, TSC, percentage of motile spermatozoa, TMS, and cell viability. Interestingly, also second level parameters showed an improvement since the percentage of TUNEL-negative and acridine orange-negative cells were significantly increased at follow-up. In addition, the percentage of cells bearing chromosomal aneuploidies, both for sexual and for autosomal chromosome, were reduced. On the other hand, varicocele correction was associated with a highly significant reduction of all US parameters, indicative of an improved testis blood flow.

All the aforementioned basal vs follow-up variations of hormonal, seminal and US parameters were maintained in both ASE and RSE patients upon stratification by scleroembolization technique, exception made for semen volume in RSE patients which was unvaried at follow-up. Importantly, no significant difference was observed between ASE and RSE patients for clinical parameters at follow-up, allowing to perform subsequent analyses on all patients as a whole group.

In order to address the possible prognostic value of demographic and clinical parameters at basal, in terms of improvement of sperm parameters associated with the treatment, patients were preliminarily stratified according to left and right testis varicocele degree scale (Figure 1A-B). Basal left testis varicocele degree greater than 2 was associated with a significant increase of the TSC at follow-up, whilst the TMS showed an increase only for degrees 2 and 3. Sperm viability showed some significant improvements only for patients with degree 3. On the other hand, basal right testis varicocele degree 0 and 1 were associated with a significant increase of the TSC. An increase of TMS was observed only for degree 0.

Patients were then stratified according to quartiles of increasing basal age, FSH, total testosterone (TT), total sperm count, type A motility and typical morphology and evaluated for the basal-to-follow-up variation of TSC and TMS (Figure 2 and 3). Varicocele correction was associated with a significant improvement of both TSC and TMS in patients relying to quartiles 1-2 of age, quartiles 3-4 of TT, quartiles 3-4 of type A motility, quartiles 2 and 4 of viability and quartiles 1 and 4 of typical sperm morphology. On the other hand, basal vs follow-up TSC was significantly increased in relating to quartiles 1-3 of FSH, whilst TMS was increased only for quartiles 1 and 2. Also patients relying to basal TSC quartiles 2-4 showed a significant increase of TSC at follow up, whilst TMS was increased only for quartiles 2-3. Finally, patients with basal type A+B motility relating to quartiles 1, 2 and 4 showed a significant increase of TSC, whilst an increase of TMS was observed only for quartile 4. Minor impact was observed on sperm viability and typical sperm morphology (Supplemental Table S1A). In particular, a significant increase of cell viability was observed for quartile 3 of basal type A motility, quartile 3 of basal type A+B motility and quartiles 1-2 of basal sperm viability. Differently a significant increase of typical sperm morphology was observed for basal quartiles 1 and 4 of typical sperm morphology.

A similar approach was applied to basal US parameters, for which higher consistency between TSC and TMS was observed (Figures 4 and 5). In fact, a significant increase of basal vs follow-up TSC and TMS emerged for patients relying in quartiles 3-4 of L-TTP, quartiles 2 and 4 of L-MTT, quartiles 3-4 of R-MTT, quartiles 2 and 4 of L-Wash IN, quartiles 3-4 of L-Wash OUT and quartiles 1 and 4 of R-Wash OUT. Only basal R-Wash IN parameter showed some difference between the two outcome parameters since patients relying in quartiles 2, 3 and 4 showed a significant increase of TSC whilst an increase of TMS was observed only for quartiles 3-4. On the other hand, a significant increase of the basal-to-follow-up sperm viability was observed for basal quartile 4 of TTP-R, quartile 4 of TTP-L and quartile 3 of MTT-L (Supplemental Table S1B).

To better evaluate the association between basal pattern quartiles and TSC and TMS, a logistic regression analysis was performed to estimate the relative impact of basal age, FSH, TT, TSC,

type A Motility, TMS, sperm viability and typical morphology, as continuous variables, in associating with TSC or TMS doubling at follow-up as dichotomous variable (Table 3A). Interestingly, basal type A motility showed an independent and significant association with both TSC and TMS doubling at follow-up (respectively, $P=0.033$, $OR=1.102$, 95% C.I. 1.008-1.205 and $P=0.047$, $OR=1.092$, 95% C.I. 1.001-1.190). A similar approach was then applied to basal US parameters as continuous variables (Table 3B), showing that L-MTT was independently and significantly associated with both TSC and TMS doubling at follow-up (respectively: $P=0.002$, $OR=1.183$, 95% C.I. 1.061-1.319 and $P=0.022$, $OR=1.125$, 95% C.I. 1.017-1.243) whilst L-Wash out was associated with TMS doubling ($P=0.019$, $OR=1.129$, 95% C.I. 1.020-1.249). Of note, neither scleroebolization procedures nor left or right varicocele grading was associated with either of the seminal outcome parameters (Supplemental Table S2A-B).

On this base, a receiver operating characteristic (ROC) analysis was performed in order to correlate type A motility and L-MTT as continuous variables with TSC doubling at follow-up and A motility, L-MTT and L-wash-out with TMS doubling (Figure 6 A, B). Only L-MTT values equal or greater than, respectively 35.95 s and 37.5 s were prognostic of TSC and TMS doubling at follow-up (respectively: $p<0.001$, $AUC=0.834$, 95% C.I. 0.747-0.921 and $P<0.001$, $AUC=0.805$, 95% C.I. 0.708-0.901). On the other hand, L-Wash OUT values equal or greater than 27.2 s were prognostic of TMS doubling ($P<0.001$, $AUC=0.779$, 95% C.I. 0.676-0.882).

Discussion

In this study we provide evidence that the treatment of varicocele with scleroembolization procedure upon appropriate patient selection, associates with a significant improvement of semen parameters and bilateral testicular microcirculation status. In addition, basal US-based testicular contrast harmonic imaging assessment showed significant prognostic value to address those patients who would benefit most from varicocele correction in terms of total sperm count and/or total motile count increase.

Available data on the possible improvement of testis function upon varicocele correction shows controversial results. Nilsson et al. reported no significant improvement of semen parameters after surgical correction compared to controls [26]. Differently, Madgar et al. demonstrated marked improvements in sperm parameters and fertility rates [27]. On the other hand, recent studies further support the beneficial effects of varicocele treatment on semen quality, since Morini et al. observed significant improvements in sperm concentration and motility, along with a decrease in sperm head and tail abnormalities after surgical correction of varicocele [28]. Similarly, Mongioi et al. found significant improvements in seminal parameters and,

importantly, showed that sclerotherapy led to enhanced spontaneous pregnancy rates compared to surgical varicocelectomy [29].

In spite of these evidence, major efforts are still required to address which basal parameters are prognostic for the indication of varicocele correction aimed at improving seminal outcome. Ory et al. showed that elevated FSH levels at basal are associated with poorer semen outcomes, emphasizing the importance of including hormonal assessments in the preoperative evaluation of patients with varicocele [30]. In addition, recent metaanalyses showed that the preoperative FSH levels and luteinizing hormone (LH)/T ratio are promising prognostic parameters to a favorable outcome after varicocele treatment [31–33]. In this study we showed that, by the use of a multivariate analysis performed on quartiles of basal demographic and clinical parameters, some significant and consistent increase of TSC and/or TMS were obtained in younger patients, with basal age < 26 years, together with FSH <8.8 IU/mL, total T>15.2 nmol/L and TSC > 7.65×10^6 cells/ejaculate. However, all these parameters failed to associate with the doubling TSC or TMS at follow-up, compared to basal, at logistic regression analysis. On the other hand, a consistent association with both TSC and TMS doubling was observed for basal L-MTT at both logistic regression and ROC analysis. In particular, a significant association with TSC and TMS doubling was observed for L-MTT values greater than, respectively, 35.95 s and 37.5 s. Strikingly, these threshold values are closed to those previously identified by Caretta et al. as predictors of low sperm count (TSC< 39×10^6 cells/ejaculate) in varicocele patients [10]. Taken together, these data highlight the role of first and second level-diagnostic work-up of patients considered eligible for varicocele treatment, in order to likely exclude other causes of alteration of semen parameters. In fact, it is recognized that severe oligozoospermia and very high levels of FSH are associated with other causes of primary testis impairment than varicocele that would poorly benefit from the treatment [34, 35].

These findings reinforce the concept that the functional status of the testicular microcirculation represents a key determinant of spermatogenic efficiency. Recent data highlight that testicular hypoxia, a recognized consequence of impaired venous drainage in varicocele, may exert multifaceted deleterious effects on germ-cell metabolism, oxidative stress, epididymal maturation and spermatogenesis [36, 37]. In particular, the review by Lord et al. highlight how hypoxia can alter gene-expression, endocrine signalling and epididymal epithelial cell function in humans and animal models, thus providing a plausible explanation for the impairment of local micro-circulation that we observed as delayed MTT at US analysis. In this frame, the prognostic significance of delayed L-MTT may reflect an underlying hypoxic microenvironment, which becomes amenable to correction by the varicocele treatment.

The correction of venous stasis is then suggested to restore physiological perfusion gradients, oxygenation, and nutrient delivery to the seminiferous epithelium, promoting a functional recovery of the germinal line.

Beyond classical semen and hormonal parameters, integrating multiple indices could allow a more precise and individualized assessment of testicular function. A multimodal prognostic model, combining hormonal, biochemical, and imaging-derived parameters, could improve the selection of candidates for varicocele treatment and provide deeper insight into its physiological basis [35]. In practice, this approach could stratify patients according to their residual testicular reserve, helping to identify those more likely to benefit from treatment. Moreover, it could provide a conceptual framework linking imaging-derived microcirculatory findings with molecular and cellular mechanisms, including hypoxia and oxidative stress.

We acknowledge several limitations for this study. Firstly, the retrospective nature of the analysis and the relatively small sample size may limit the generalizability of our findings. We also excluded patients featured by unsuccessful scleroembolization procedures, thus missing to evaluate the reasons for failure that may provide additional insights, particularly with regard to technical aspects and procedural feasibility. In addition, we adopted TSC and TMS doubling to measure the semen outcome of varicocele treatment. As per standard practice, patient's data on fertility outcomes were not available and data on seminal outcomes were used as valuable surrogates. In addition, whilst possibly questionable, the use of TSC and TMS doubling as endpoints is consistent with previous studies on this topic and can be considered clinically sound in a real-life scenario in order to provide the patient with a realistic perspective of the possible improvement of seminal parameters during pre-interventional counseling [38–41].

Conclusions

In this study we showed that in well selected patients varicocele scleroembolization has beneficial effects on semen outcome and testis vascularization at ultrasound analysis. Although the overall characterization of the patient remains a key point for the eligibility for treatment, some parameters such as baseline sperm count and reduced testicular perfusion represent important clinical descriptors for the identification those subjects who can benefit most from the treatment.

Author's Contribution.

Conceptualization: Luca De Toni, Giordana Ferraioli, Andrea Garolla. Methodology: Giorgio De Conti, Massimo Iafrate, Fabrizio Dal Moro, Alberto Ferlin. Software: Pierfrancesco Palego, Andrea Graziani, Nicola Caretta. Validation: Pierfrancesco Palego, Andrea Graziani, Nicola Caretta. Formal analysis: Luca De Toni, Giordana Ferraioli. Investigation and data: Giordana Ferraioli, Matteo Todisco, Sara Congiu, Pierfrancesco Palego, Andrea Graziani, Nicola Caretta. Original draft preparation: Luca De Toni, Giordana Ferraioli. Review, and editing: Giuseppe Grande, Andrea Garolla

403

404

405 References

- 406 1. Dubin L, Amelar RD (1971) Etiologic Factors in 1294 Consecutive Cases of Male
407 Infertility. *Fertil Steril* 22:469–474. [https://doi.org/10.1016/S0015-0282\(16\)38400-X](https://doi.org/10.1016/S0015-0282(16)38400-X)
- 408 2. Alsaikhan B, Alrabeeah K, Delouya G, Zini A (2016) Epidemiology of varicocele. *Asian*
409 *J Androl* 18:179. <https://doi.org/10.4103/1008-682X.172640>
- 410 3. Jensen CFS, Østergren P, Dupree JM, et al (2017) Varicocele and male infertility. *Nat*
411 *Rev Urol* 14:523–533. <https://doi.org/10.1038/nrurol.2017.98>
- 412 4. Ferlin A, Calogero AE, Krausz C, et al (2022) Management of male factor infertility:
413 position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS).
414 *J Endocrinol Invest* 45:1085–1113. <https://doi.org/10.1007/s40618-022-01741-6>
- 415 5. Bellastella G, Carotenuto R, Caiazza F, et al (2022) Varicocele: An Endocrinological
416 Perspective. *Frontiers in Reproductive Health* 4:.
417 <https://doi.org/10.3389/frph.2022.863695>
- 418 6. Agarwal A, Sharma R, Harlev A, Esteves S (2016) Effect of varicocele on semen
419 characteristics according to the new 2010 World Health Organization criteria: a
420 systematic review and meta-analysis. *Asian J Androl* 18:163.
421 <https://doi.org/10.4103/1008-682X.172638>
- 422 7. Garolla A, Torino M, Miola P, et al (2015) Twenty-four-hour monitoring of scrotal
423 temperature in obese men and men with a varicocele as a mirror of spermatogenic
424 function. *Human Reproduction* 30:1006–1013. <https://doi.org/10.1093/humrep/dev057>
- 425 8. Russo GI, Saleh R, Finocchi F, et al (2024) Impact of Varicocele on Testicular Oxidative
426 Stress and Sperm Parameters in Experimental Animals: A Systematic Review and Meta-
427 Analysis. *World J Mens Health* 42:.
<https://doi.org/10.5534/wjmh.230260>
- 428 9. James Wright E, Young GPH, Goldstein M (1997) Reduction in testicular temperature
429 after varicocelectomy in infertile men. *Urology* 50:257–259.
430 [https://doi.org/10.1016/S0090-4295\(97\)00191-X](https://doi.org/10.1016/S0090-4295(97)00191-X)
- 431 10. Caretta N, Palego P, Schipilliti M, et al (2010) Testicular Contrast Harmonic Imaging to
432 Evaluate Intratesticular Perfusion Alterations in Patients With Varicocele. *Journal of*
433 *Urology* 183:263–269. <https://doi.org/10.1016/j.juro.2009.08.140>
- 434 11. Aua A Report on Vari cocel e and Inferti l i ty Archived Document-For Reference Only
- 435 12. Jungwirth A, Giwercman A, Tournaye H, et al (2012) European association of urology
436 guidelines on male infertility: The 2012 update. *Eur Urol* 62:324–332.
437 <https://doi.org/10.1016/j.eururo.2012.04.048>
- 438 13. Wang Q, Yu Y, Liu Y, Wang L (2019) Outcome of varicocelectomy on different degrees
439 of total motile sperm count: A systematic review and meta-analysis. *Syst Biol Reprod*
440 *Med* 65:430–436. <https://doi.org/10.1080/19396368.2019.1655813>
- 441 14. Graziani A, Rocca MS, Vinanzi C, et al (2024) Genetic Causes of Qualitative Sperm
442 Defects: A Narrative Review of Clinical Evidence. *Genes (Basel)* 15:600.
443 <https://doi.org/10.3390/genes15050600>

- 444 15. Grande G, Graziani A, Ferlin A (2024) Guideline for unexplained couple infertility:
445 misunderstandings on the approach to the male factor. *Human Reproduction* 39:859–
446 860. <https://doi.org/10.1093/humrep/deae032>
- 447 16. (2013) World Medical Association Declaration of Helsinki. *JAMA* 310:2191.
448 <https://doi.org/10.1001/jama.2013.281053>
- 449 17. WHO laboratory manual for the examination and processing of human semen Sixth
450 Edition
- 451 18. Jungwirth A, Giwercman A, Tournaye H, et al (2012) European Association of Urology
452 Guidelines on Male Infertility: The 2012 Update. *Eur Urol* 62:324–332.
453 <https://doi.org/10.1016/j.eururo.2012.04.048>
- 454 19. Schlegel PN, Sigman M, Collura B, et al (2021) Diagnosis and Treatment of Infertility in
455 Men: AUA/ASRM Guideline PART II. *Journal of Urology* 205:44–51.
456 <https://doi.org/10.1097/JU.0000000000001520>
- 457 20. Keene DJB, Cervellione RM (2017) Antegrade sclerotherapy in adolescent varicocele
458 patients. *J Pediatr Urol* 13:305.e1-305.e6. <https://doi.org/10.1016/j.jpuro.2016.12.018>
- 459 21. Di Bisceglie C, Fornengo R, Grosso M, et al (2003) Follow-up of varicocele treated with
460 percutaneous retrograde sclerotherapy: Technical, clinical and seminal aspects. *J*
461 *Endocrinol Invest* 26:1059–1064. <https://doi.org/10.1007/BF03345250>
- 462 22. Garolla A, Ghezzi M, Cosci I, et al (2017) FSH treatment in infertile males candidate to
463 assisted reproduction improved sperm DNA fragmentation and pregnancy rate.
464 *Endocrine* 56:416–425. <https://doi.org/10.1007/s12020-016-1037-z>
- 465 23. Lotti F, Frizza F, Balercia G, et al (2021) The European Academy of Andrology (EAA)
466 ultrasound study on healthy, fertile men: Scrotal ultrasound reference ranges and
467 associations with clinical, seminal, and biochemical characteristics. *Andrology* 9:559–
468 576. <https://doi.org/10.1111/andr.12951>
- 469 24. Bertolotto M, Freeman S, Richenberg J, et al (2020) Ultrasound evaluation of
470 varicoceles: systematic literature review and rationale of the ESUR-SPIWG Guidelines
471 and Recommendations. *J Ultrasound* 23:487–507. <https://doi.org/10.1007/s40477-020-00509-z>
- 473 25. Geis S, Gehmert S, Lamby P, et al (2012) Contrast enhanced ultrasound (CEUS) and
474 time intensity curve (TIC) analysis in compartment syndrome: First results. *Clin*
475 *Hemorheol Microcirc* 50:1–11. <https://doi.org/10.3233/CH-2011-1438>
- 476 26. NILSSON S, EDVINSSON A, NILSSON B (1979) Improvement of Semen and
477 Pregnancy Rate after Ligation and Division of the Internal Spermatic Vein: Fact or
478 Fiction? *Br J Urol* 51:591–596. <https://doi.org/10.1111/j.1464-410X.1979.tb03609.x>
- 479 27. Madgar I, Weissenberg R, Lunenfeld B, et al (1995) Controlled trial of high spermatic
480 vein ligation for varicocele in infertile men. *Fertil Steril* 63:120–124.
481 [https://doi.org/10.1016/S0015-0282\(16\)57306-3](https://doi.org/10.1016/S0015-0282(16)57306-3)
- 482 28. Morini D, Spaggiari G, Daolio J, et al (2021) Improvement of sperm morphology after
483 surgical varicocele repair. *Andrology* 9:1176–1184. <https://doi.org/10.1111/andr.13012>
- 484 29. Mongioi LM, Mammino L, Compagnone M, et al (2019) Effects of Varicocele Treatment
485 on Sperm Conventional Parameters: Surgical Varicocelectomy Versus Sclerotherapy.
486 *Cardiovasc Intervent Radiol* 42:396–404. <https://doi.org/10.1007/s00270-018-2136-4>

30. Ory J, Tradewell MB, Blankstein U, et al (2022) Artificial Intelligence Based Machine Learning Models Predict Sperm Parameter Upgrading after Varicocele Repair: A Multi-Institutional Analysis. *World J Mens Health* 40:618. <https://doi.org/10.5534/wjmh.210159>
31. Mei Y, Ji N, Feng X, et al (2024) Don't wait any longer, conceive in time: a systematic review and meta-analysis based on semen parameters after varicocelectomy. *Int Urol Nephrol*. <https://doi.org/10.1007/s11255-024-04080-y>
32. Cannarella R, Shah R, Hamoda TA-AA-M, et al (2024) Does Varicocele Repair Improve Conventional Semen Parameters? A Meta-Analytic Study of Before-After Data. *World J Mens Health* 42:92. <https://doi.org/10.5534/wjmh.230034>
33. Ok F, Erdogan O, Durmus E (2020) Can preoperative gonadotropin and testosterone levels predict the success of varicocelectomy? *Andrologia* 52:.. <https://doi.org/10.1111/and.13887>
34. Crafa A, Russo M, Cannarella R, et al (2024) Predictability of varicocele repair success: preliminary results of a machine learning-based approach. *Asian J Androl*. <https://doi.org/10.4103/aja202438>
35. Crafa A, Cannarella R, Condorelli RA, et al (2024) Predictive parameters of the efficacy of varicocele repair: a review. *Asian J Androl*. <https://doi.org/10.4103/aja202420>
36. Aitken RJ, Curry BJ (2011) Redox Regulation of Human Sperm Function: From the Physiological Control of Sperm Capacitation to the Etiology of Infertility and DNA Damage in the Germ Line. *Antioxid Redox Signal* 14:367–381. <https://doi.org/10.1089/ars.2010.3186>
37. Aitken RJ (2011) The Capacitation-Apoptosis Highway: Oxysterols and Mammalian Sperm Function. *Biol Reprod* 85:9–12. <https://doi.org/10.1095/biolreprod.111.092528>
38. Selice R, Garolla A, Pengo M, et al (2011) The response to FSH treatment in oligozoospermic men depends on FSH receptor gene polymorphisms. *Int J Androl* 34:306–312. <https://doi.org/10.1111/j.1365-2605.2010.01086.x>
39. Garolla A, Selice R, Engl B, et al (2014) Spermatid count as a predictor of response to FSH therapy. *Reprod Biomed Online* 29:102–112. <https://doi.org/10.1016/j.rbmo.2014.02.014>
40. Foresta C, Bettella A, Merico M, et al (2000) FSH in the treatment of oligozoospermia. *Mol Cell Endocrinol* 161:89–97. [https://doi.org/10.1016/S0303-7207\(99\)00228-2](https://doi.org/10.1016/S0303-7207(99)00228-2)
41. Foresta C, Bettella A, Ferlin A, et al (1998) Evidence for a Stimulatory Role of Follicle-Stimulating Hormone on the Spermatogonial Population in Adult Males. *Fertil Steril* 69:636–642. [https://doi.org/10.1016/S0015-0282\(98\)00008-9](https://doi.org/10.1016/S0015-0282(98)00008-9)

Figure Captions

Figure 1

Multivariate analysis of basal-to-follow-up comparison of total sperm count and total motile sperm count (TMS) in 85 patients undergone to varicocele scleroembolization treatment, stratified according to basal left (A) and right (B) varicocele degree score according to the Penile Imaging Working Group of the European Society of Urogenital Radiology (ESUR-

SPIWG) and the European Academy of Andrology [24]. Significance: comparison P values between conditions are indicated

Figure 2

Multivariate analysis of basal-to-follow-up comparison of total sperm count and total motile sperm count (TMS) in 85 patients undergone to varicocele scleroembolization treatment, stratified according to basal quartiles of age, follicle stimulating hormone (FSH), total testosterone and total sperm count values. Significance: comparison P values between conditions are indicated.

Figure 3

Multivariate analysis of basal-to-follow-up comparison of total sperm count and total motile sperm count (TMS) in 85 patients undergone to varicocele scleroembolization treatment, stratified according to basal quartiles of type A sperm motility, type A+B sperm motility, sperm viability and tipycoal sperm morphology. Significance: comparison P values between conditions are indicated.

Figure 4

Multivariate analysis of basal-to-follow-up comparison of total sperm count and total motile sperm count (TMS) in 85 patients undergone to varicocele scleroembolization treatment, stratified according to basal quartiles of ultrasound left (L) and right (R) time to peak (TTP) and mean transit time (MTT). Significance: comparison P values between conditions are indicated.

Figure 5

Multivariate analysis of basal-to-follow-up comparison of total sperm count and total motile sperm count (TMS) in 85 patients undergone to varicocele scleroembolization treatment, stratified according to basal quartiles of ultrasound left (L) and right (R) wash in time and wash out time. Significance: comparison P values between conditions are indicated.

Figure 6

Receiver Operating Characteristic curve analysis of the association between basal type A sperm motility, left ultrasound mean transit time (L-MTT) and left ultrasound wash out time with total sperm count or total motile sperm count doubling after varicocele correction by scleroembolization technique.