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Effects of Varicocele Repair on Sperm DNA Fragmentation and Seminal Malondialdehyde Levels in Infertile Men with Clinical Varicocele: A Systematic Review and Meta-Analysis

Rossella Cannarella[1,2,3], Rupin Shah[1,3,4], Ramadan Saleh[3,5], Florence Boitrelle[3,6,7], Taha Abo-Almagd Abdel-Meguid Hamoda[3,8,9], Rajender Singh[3,10], Gianmaria Salvio[3,11], Tuncay Toprak[3,12], Marco Falcone[3,13], Murat Gul[3,14], Fotios Dimitriadis[3,15], Amarnath Rambhatla[3,16], Giorgio I. Russo[3,17], Edmund Ko[3,18], Armand Zini[3,19], Parviz Kavoussi[3,20], Nguyen Ho Vinh Phuoc[3,21,22], Hussein Kandil[3,23], Ramy Abou Ghayda[3,24], Ponco Birowo[3,25], Nazim Gherabi[3,26], Erman Ceyhan[3,27], Jie Dong[3,28], Vineet Malhotra[3,29], Damayanthi Durairajanayagam[3,30], Bircan Kolbasi[3,31], Fahmi Bahar[3,32], Gokhan Calik[3,33], Selahittin Çayan[3,34], Germar-Michael Pinggera[3,35], Aldo E. Calogero[2,3], Osvaldo Rajmil[3,36,37], Taymour Mostafa[3,38], Widi Atmoko[3,25], Ahmed M. Harraz[3,39,40,41], Tan V. Le[3,21,22], Jean de la Rosette[3,42], Lukman Hakim[3,43], Edoardo Pescatori[3,44], Oleg Sergeyev[3,45,46], Ayman Rashed[3,47], Pallavi Saini[10], Ashok Agarwal[3,48]

[1]Department of Urology, Glickman Urological & Kidney Institute, Cleveland Clinic Foundation, Cleveland, OH, USA, [2]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy, [3]Global Andrology Forum, Moreland Hills, OH, USA, [4]Department of Urology, Lilavati Hospital and Research Centre, Mumbai, India, [5]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, Egypt, [6]Reproductive Biology, Fertility Preservation, Andrology, CECOS, Poissy Hospital, Poissy, [7]Department of Biology, Reproduction, Epigenetics, Environment and Development, Paris Saclay University, UVSQ, INRAE, BREED, Jouy-en-Josas, France, [8]Department of Urology, King Abdulaziz University, Jeddah, Saudi Arabia, [9]Department of Urology, Faculty of Medicine, Minia University, Minia, Egypt, [10]Division of Endocrinology, Central Drug Research Institute, Lucknow, India, [11]Department of Endocrinology, Polytechnic University of Marche, Ancona, Italy, [12]Department of Urology, Fatih Sultan Mehmet Training and Research Hospital, University of Health Sciences, Istanbul, Turkey, [13]Department of Urology, Molinette Hospital, A.O.U. Città della Salute e della Scienza, University of Turin, Torino, Italy, [14]Department of Urology, Selçuk University School of Medicine, Konya, Turkey, [15]Department of Urology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece, [16]Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI, USA, [17]Urology Section, University of Catania, Catania, Italy, [18]Department of Urology, Loma Linda University Health, Loma Linda, CA, USA, [19]Department of Surgery, McGill University, Montreal, QC, Canada, [20]Department of Reproductive Urology, Austin Fertility & Reproductive Medicine/Westlake IVF, Austin, TX, USA, [21]Department of Andrology, Binh Dan Hospital, Ho Chi Minh City, [22]Department of Andrology and Nephro-Urology, Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam, [23]Fakih IVF Fertility Center, Abu Dhabi, UAE, [24]Urology Institute, University Hospitals, Case Western Reserve University, Cleveland, OH, USA, [25]Department of Urology, Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [26]Department of Medicine, Algiers University, Algiers, Algeria, [27]Department of Urology, Faculty of Medicine, Baskent University, Ankara, Turkey, [28]Reproductive Medical Center, Tangdu Hospital, The Fourth Military Medical University, Xi'an, Shaanxi Province, China, [29]Department of Urology, SCM Clinic and Hospital, New Delhi, India, [30]Department of Physiology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia, [31]Department of Histology and Embryology, School of Medicine, Istanbul Medipol University, Istanbul, Turkey, [32]Andrology Section, Siloam Sriwijaya Hospital, Palembang, Indonesia, [33]Department of Urology, Istanbul Medipol University, Istanbul, [34]Department of Urology, University of Mersin School of Medicine, Mersin, Turkey, [35]Department of Urology, Medical University Innsbruck, Innsbruck, Austria, [36]Andrology Department, Fundació Puigvert, Barcelona, [37]Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Universitat Autònoma de Barcelona, Barcelona, Spain, [38]Department of Andrology, Sexology and

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Correspondence to: Ashok Agarwal https://orcid.org/0000-0003-0585-1026
Global Andrology Forum, 130 West Juniper Lane, Moreland Hills, OH 44022, USA.
Tel: +1-216-312-5829, **E-mail:** agarwaa32099@outlook.com, **Website:** https://www.globalandrologyforum.com

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STIs, Faculty of Medicine, Cairo University, Cairo, Egypt, [39]Department of General Surgery, Urology Unit, Farwaniya Hospital, Farwaniya, [40]Department of Urology, Sabah Al Ahmad Urology Center, Kuwait City, Kuwait, [41]Department of Urology, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt, [42]Department of Urology, Istanbul Medipol Mega University Hospital, Istanbul, Turkey, [43]Department of Urology, Universitas Airlangga Teaching Hospital, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [44]Andrology and Reproductive Medicine Unit, Next Fertility GynePro, Bologna, Italy, [45]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, [46]Laboratory of Genetics of Reproductive Disorders, Research Centre for Medical Genetics, Moscow, Russia, [47]Department of Urology, 6th October University, Tanta, Egypt, [48]Cleveland Clinic, Cleveland, OH, USA

Purpose: Varicoceles can be a source of elevated seminal oxidative stress (OS) and sperm DNA fragmentation (SDF). However, it remains unclear whether varicocele repair (VR) could reduce these parameters. This systematic review and meta-analysis (SRMA) aims to investigate the impact of VR on SDF and seminal malondialdehyde (MDA).

Materials and Methods: A literature search was performed in Scopus, PubMed, Ovid, Embase, and Cochrane databases. This SRMA included randomized controlled trials and observational studies reporting the pre- and postoperative levels of SDF and seminal OS in infertile men with clinical varicocele that underwent VR. Subgroup analyses included techniques of VR and SDF testing. The effect size was expressed as standardized mean difference (SMD).

Results: Out of 1,632 abstracts assessed for eligibility, 29 studies with 1,491 infertile men were included. The analysis showed a significant reduction in SDF after VR, compared to preoperative values (SMD –1.125, 95% confidence interval [CI] –1.410, –0.840; p<0.0001) with high inter-study heterogeneity (I^2=90.965%). Reduction in SDF was evident with microsurgical technique and non-microsurgical inguinal approaches (SMD –1.014, 95% CI –1.263, –0.765; p<0.0001, and SMD –1.495, 95% CI –2.116, –0.873; p<0.0001), respectively. Reduction in SDF was significant irrespective of testing was done by sperm chromatin dispersion (SMD –2.197, 95% CI –3.187, –1.207; p<0.0001), sperm chromatin structure assay (SMD –0.857, 95% CI –1.156, –0.559; p<0.0001) or TUNEL (SMD –1.599, 95% CI –2.478, –0.719; p<0.0001). A significant decrease in seminal MDA levels was observed following VR (SMD –2.450, 95% CI –3.903 to –0.997, p=0.001) with high inter-study heterogeneity (I^2=93.7%).

Conclusions: Using pre- and post-intervention data, this SRMA indicates a significant reduction in SDF and seminal MDA levels in infertile men with clinical varicocele treated with VR. These findings may have important implications for the future management of this selected group of infertile patients.

Keywords: Infertility, male; Malondialdehyde; DNA fragmentation; Varicocele

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INTRODUCTION

Infertility is defined as the inability of a couple to conceive a child after one year of regular, unprotected sexual intercourse [1]. It has been recognized as a universal public health issue by the World Health Organization (WHO), with current evidence indicating 9% prevalence and 56% of couples seeking medical care [2]. An estimated 48.5 million couples that have unprotected sexual intercourse suffer from infertility worldwide [3], with a male factor solely contributing to approximately 2.5% to 12% of couple infertility around the world [4].

Although semen analysis represents a crucial investigation in the infertile male, it remains sub-optimal in the evaluation and management of male infertility, with serious concerns regarding its validity in discerning between fertile and infertile men [5,6]. Sperm DNA fragmentation (SDF) has emerged as a useful tool in offering a more functional assessment of male fertility [7,8]. SDF is a marker of damaged chromatin in spermatozoa. Chromatin damage may include a broad spectrum of DNA defects such as single or double DNA strand breaks, base deletions or modifications, inter/intra-strand DNA cross-linkage, or protamine deficiency [9]. The rationale for assessing DNA integrity resides in the crucial role of chromatin compaction and structural stability in producing a fully functional sperm cell [10-13]. The recent WHO Laboratory Manual for the Examination and Processing of Human Semen 6th edition has subsequently dedicated an extensive narrative highlighting the importance of SDF assessment in

male infertility management [14].

Several studies have demonstrated the association between elevated SDF and failure of natural pregnancy [13,15-17]. In a systematic review and meta-analysis (SRMA) on males from couples suffering from recurrent idiopathic miscarriage, SDF levels were reported to be higher compared to that of fertile controls (mean difference [MD]=11.98%, 95% confidence interval [CI] 6.64-17.32 p<0.001) [18]. Additionally, in the context of assisted reproductive technology (ART), SDF has been recognized as a factor that correlates with failed intrauterine insemination (IUI) [19-21].

SDF is considered an indirect marker of oxidative stress (OS). OS is defined as the imbalance between the generation of reactive oxygen species (ROS) and the scavenger capacity. While low levels of ROS are needed for sperm development and for acquiring their fertilizing capacity, high levels or impairment of sperm antioxidant systems result in OS and infertility [22]. Spermatozoa are particularly vulnerable to OS, as ROS can disrupt the sperm cell membrane by lipoperoxidation and can lead to sperm protein and DNA damage. Seminal malondialdehyde (MDA) is a direct biomarker of OS, which reflects the degree of sperm cell lipoperoxidation by ROS and is paralleled with an increase of SDF and impairment of sperm motility [23]. Interestingly, sperm lipid peroxidation has been questioned as possibly involved in recurrent pregnancy loss (RPL). A study on 16 RPL cases indeed reported significantly higher levels of MDA in the patient group compared to fertile healthy controls [24], thus configuring a possible future role of this test in the diagnosis of male infertility. Varicocele is one of the most common correctable causes of male infertility, which is found in around 15% of healthy men, 35%–44% of men with primary infertility, and up to 45%–80% of men with secondary infertility [25-27]. Its prevalence rises with age and most varicocele develops during puberty [28,29]. Varicocele can cause male infertility through numerous mechanisms. Impairment of venous drainage may increase OS, increase scrotal temperature, induce testicular hypoxia, and cause abnormal reflux of metabolites [30]. Varicocele can cause up to 2.6 ℃ temperature elevation in the scrotum [31]. Increasing scrotal temperature has a detrimental effect on spermatogenesis, including increased SDF and impaired sperm fertilization capability [32].

Varicoceles are associated with increased OS which can lead to DNA damage in sperm [33]. Approximately 50% of men with clinically palpable varicocele have elevated SDF, and varicocele repair (VR) is associated with a decrease in SDF, especially in higher-grade varicoceles [34]. The reduction in SDF after VR may result in higher clinical pregnancy rates [35]. Despite evidence linking elevated SDF with varicoceles and improvement in SDF after VR, there is a paucity of recommendations in the major male infertility guidelines regarding VR in patients with a palpable varicocele and elevated SDF, as well as scenarios on when to obtain SDF testing.

Whether VR can influence seminal OS markers—particularly seminal MDA—is unknown. A better understanding of the impact of VR on SDF and OS—beyond the bulk semen parameters—can help guide the treatment of infertile men with varicoceles. The aim of this study is to conduct a SRMA to examine the impact of VR on SDF values as well as seminal MDA levels in infertile men.

MATERIALS AND METHODS

1. Search strategy

This meta-analysis was performed following the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines [36] and the Preferred Reporting Items for SRMA Protocols (PRISMA-P) guidelines [37]. The data were extracted through extensive search in the Scopus, PubMed, Embase and Cochrane databases considering publications from the 1970s to December 2022. The search strategy included the combination of the following Medical Subjects Headings (MeSH) terms and keywords: varicoc*, management, embolization, mbolization, microsurg*, varicocelectomy, repair, correction, treatment, ligation, surg*, operation, radiolog*, sperm*, semen, seminal, ejaculate, asperm*, azoo*, azoosperm*, oligo*, oligosperm*, oligozoosperm*, astheno*, asthenosperm*, asthenozoosperm*, terato*, teratosperm*, teratozoosperm*, and necro*, necrosperm*, necrozoosperm*, sperm DNA fragmentation, SDF, DNA fragmentation index, DFI. The search was restricted to human studies and no language limitations were applied. The studies were initially assessed for inclusion by reading their titles and abstracts. When the abstract did not immediately reveal whether the study contained data relevant to our meta-analysis, the full text was read carefully. The evaluation for inclusion of

the studies was performed by a team of 49 researchers selected after a training period, to allow an objective approach. In detail, each article was independently assessed for eligibility by two different researchers. Any disagreement was resolved by a third author. The protocol of this SRMA has been registered in the PROSPERO database (CRD42022329848).

2. Selection criteria

This review included all studies on infertile males with clinical varicocele until December 2022 that measured SDF, and included data on seminal MDA levels when available, in varicocele treated patients, whose pre-treatment values are used as control. All the eligible studies were chosen following the Population, Intervention, Comparison/Comparator, Outcomes, and Study type (PICOS) model (Supplement Table 1) [38]. Studies including adolescents, studies of subclinical varicocele, studies with no VR, *in vitro* studies, animal studies, case reports, case series, communications, and studies in which the control group received any treatment were excluded.

3. Data extraction

Data were extracted from the selected studies by 49 trained researchers distributed among four teams [39]. They worked in pairs, and a third senior author resolved disagreements. The following information was obtained from each study: authors, year of publication, study design, number of varicocele patients, assays used to assess sperm DNA integrity, type of VR, follow-up period, mean SDF value before treatment, mean SDF value after treatment, mean MDA seminal level before treatment and mean MDA seminal level after treatment. When all researchers had completed their extractions, we generated a combined database, and discrepancies were resolved by consensus among extractors under the control of the leader of each team.

4. Quality assessment

The quality of evidence (QoE) assessment aims to evaluate whether each article reports high-quality data. The QoE assessment of the articles was done by the Cambridge Quality Checklists [40]. In-depth, three checklists are devised for recognizing robust studies pertaining to associations, risk factors, and causal risk factors. The correlation checklist assesses sample size and outcome measurement quality. The risk factor checklist awards high scores to studies with suitable chronological data. The causal risk factor checklist evaluates study design. Strong inferences necessitate elevated scores across corresponding checklists: for associations, risk factors, and causal risk factors. Researchers double-checked the QoE assessment of all included articles. For each article, a screener made the initial assessment of QoE scores, a verifier checked the screener's assessment, and a third expert affirmed the final agreed score.

5. Statistical analysis

1) Quantitative data analysis

For the meta-analysis, the Comprehensive Meta-Analysis software (version-2; Biostat Inc) was used. Both the standard mean difference (SMD) and the MD were used as the measure of effect size for statistical comparison between preoperative and postoperative values. To check the heterogeneity across pooled studies, the heterogeneity index (I^2) was used. I^2 is the representation of heterogeneity and it generally lies between 0 and 100%, where <50% represents low-to-moderate heterogeneity and >50% suggests high heterogeneity. Both the fixed effect and random effects models were used to calculate the pooled effect size. In the case of low heterogeneity, the fixed effect model was used to draw the statistical inference. In the case of >50% heterogeneity, the random effects model was acquired. For generating high-resolution forest plots, the SMD and CI were considered for both the "all studies" and the pooled data [41]. Additionally, we performed a meta-regression analysis to evaluate the effect of preoperative SDF rates on SDF differences before and after VR.

2) Sensitivity analysis

The method of one study removal at a time was used for sensitivity analysis. One study was excluded at a time and their pooled effect size and CI were calculated to determine if that study was sensitive. If the exclusion of a particular study resulted in a change of the inference, it was labeled as a 'sensitive study'.

3) Publication bias analysis

Asymmetrical funnel plots were taken to qualitatively suggest the presence of publication bias. The presence of publication bias represents the missing studies

from one side of the graph. We further used Egger's intercept test, for representing publication bias quantitatively.

RESULTS

Using the aforementioned search strategy, 1,632 abstracts were extracted. After the elimination of 665 duplicates, 967 abstracts were assessed. Of these, 96 articles were identified by title and abstract as review articles, case reports, book chapters, papers on different topics, and were deemed not eligible. Of the remaining 871 articles, 164 full-texts were not found, and after reading the full-text, an additional 678 papers were excluded because they contained non-extractable data, and 524 for reporting different outcomes. Finally, 29 studies assessing the impact of VR on SDF and MDA were included in the present analysis (Fig. 1) [34,42-69].

Among the 29 included studies, 23 reported data on SDF, 5 reported MDA levels and one study reported both SDF and MDA levels before and after VR (Table 1). In three studies [50,53,55], the results were subdivided by grade of varicocele, in one study, by laterality of VR [51], in one study by different time to evaluation [64], and in one study the patients were subclassified after VR as responders or non-responders [34]. The main characteristics of the included studies are shown in Table 1. The QoE assessment is shown in Table 2.

1. Sperm DNA fragmentation

A total of 23 studies reported the SDF differences between before and after VR. Twenty-three studies showed that there was a significant change in SDF in varicocele patients after treatment. Overall, the random effect model (heterogeneity: I^2=90.965%) revealed that the SDF was significantly reduced after VR (SMD: –1.256; 95% CI –1.529, –0.982; p<0.0001; MD: –6.924; 95% CI –8.438, –5.410; p<0.001) (Fig. 2).

Sensitivity analysis was performed, but no study was sensitive enough to change the conclusion that VR significantly decreases the SDF in the random model (Supplement Fig. 1). Publication bias was not apparent for all studies in the meta-analyses of SDF based on funnel plot and Egger's test (p=0.298) (Supplement Fig. 2).

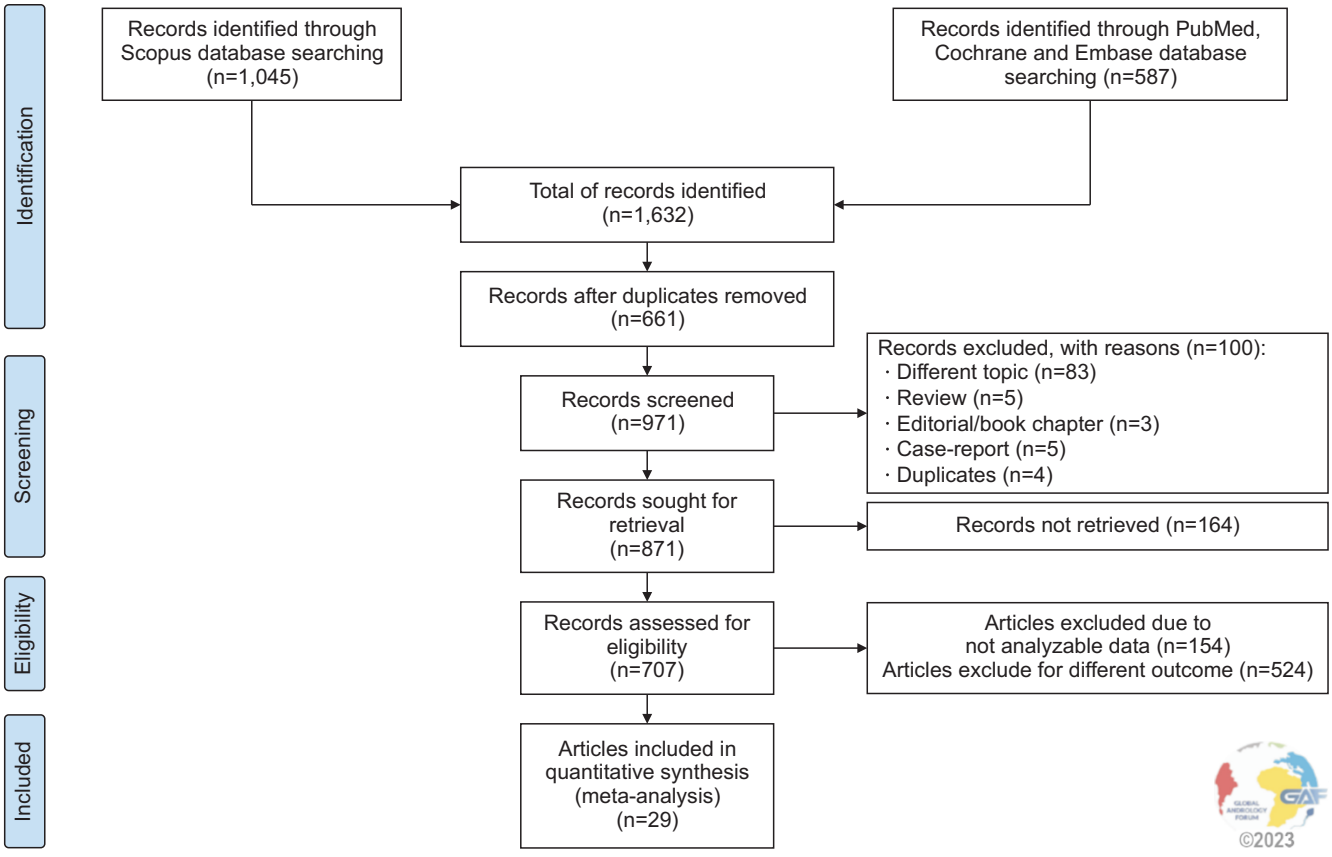

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) flow-chart for inclusion/exclusion of studies.

Table 1. Main characteristics of the included studies

First author	Year	Study design	Varicocele		Varicocele repair		Time to evaluation (mo)	Patients (n)	SDF (%)		MDA (nmol/mL)	
			Side	Grade	Type	Side			Before	After	Before	After
Kavoussi [42]	2021	Observational	-	-	MS	-	3	121	35.3±11.6	19.6±5.3		
Fathi [43]	2021	Observational	-	-	MS	-	6	45	34.93±5.56	25.75±5.15		
Abbasi [44]	2020	Observational	-	-	MS	-	3	22	13.65±11.44	10.38±4.64		
Kamal [45]	2020	Observational	-	-	NMI	-	6	34			1.9±0.6	1.1±0.3
Omar [46]	2020	Observational	-	-	MS	-	3	100			7.7±1.1	2.2±0.6
Mostafa [47]	2020	Observational	-	-	NMI	-	3	30			3.4±1.3	1.9±0.7
Camargo [48]	2019	Observational	-	-	MS	-	12	25	43.4±22.62	32.9±8.87		
Afsin [49]	2018	Observational	-	-	-	-	12	40	20.57±4.6	15.3±3.63		
Vahidi [50]	2018	Observational	Left	-	MS	-	3	30	15.93±4.96	10.86±4.44		
				1	MS	-	3	5	15.4±9.42	5.2±1.09		
				2	MS	-	3	13	16±3.39	12.07±4.71		
				3	MS	-	3	12	16.08±4.46	11.91±3.14		
Sun [51]	2018	Observational	Bilateral	-	MS	Bilateral	12	179	21.6±7.1	11.8±6		
						Left	12	179	23±8.1	12.1±6.8		
Lu [52]	2018	Observational	Left	-	MS	Left	6	27			1.75±0.22	0.58±0.06
Zaazaa [53]	2018	RCT	-	-	MS	-	3	40	34.6±4.1	28.3±5.2		
				2	MS	-	3	14	32.7±1.6	28±2.3		
				3	MS	-	3	26	35.5±4.7	28.5±6.3		
Abdelbaki [54]	2017	Observational	-	-	NMI	-	6	55	29.49±8.58	18.78±7.23		
Ni [55]	2016	Observational	-	1	MS	-	6	19	23.56±7.55	19.54±5.48	12.18±4.86	8.76±2.73
				2	MS	-	6	18	27.75±9.05	22.38±4.54	14.12±5.42	9.71±2.83
				3	MS	-	6	14	30.03±8.27	21.82±5.95	15.86±6.78	9.5±3.28
Telli [56]	2015	Observational	-	-	NMI	-	3	72	34.5±3.3	28.2±3.5		
Tavalaee [57]	2015	Observational	1	-	-	Left	3	23	15.9±1.2	10.8±1.1		
Mohammed [58]	2015	Observational	-	-	NMI	-	6	75	32.4±7.4	20±4.1		
Smit [59]	2013	Observational	-	-	-	-	3	49	35.2±13.1	30.2±14.7		
Li [60]	2012	Observational	-	-	MS	-	3	19	28.4±15.6	22.4±12.9		
Gabriel [61]	2012	Observational	-	-	MS	-	4	14	22.4±11.9	12.6±6		
La Vignera [62]	2012	Observational	Left	3	MS	Left	4	30	5±3	2.1±0.4		
Sadek [63]	2011	Observational	Bilateral	-	NMI	-	3	72	65.15±20.97	51.9±15.2		
Zini [64]	2011	Observational	-	-	MS	-	4-6	25	18±11	11±6		
							4	19	18±11	10±5		
							6	19	18±11	7±3		
Ghazi [65]	2011	Observational	Bilateral	-	MS	Bilateral	6	82	21.5±11.2	13±14.3		
Dada [66]	2010	Observational	-	-	-	-	6	11	60.82±7.12	32.58±6.12		
Nasr Esfahani [67]	2010	Observational	-	3	-	-	6	70	45.69±2.43	35.9±2.4		
Smit [34]	2010	Observational	-	-	-	-	3	31[a]	35.3±14.3	28.6±14.7		
								18[b]	35±11.2	33±14.8		
Zini [68]	2005	Observational	-	-	MS	-	6	37	27.7±17.64	24.6±16.42		
Yeşilli [69]	2005	Observational	-	-	MS	-	6	26			0.61±0.09	0.58±0.06

Values are presented as mean±standard deviation.
MDA: malondialdehyde, MS: microsurgical varicocele repair, NMI: non-microsurgical inguinal varicocele repair, RCT: randomized controlled trial, SDF: sperm DNA fragmentation.
[a]Responders to varicocele repair (improvement >50% in sperm concentration), [b]Non-responders to varicocele repair.

Table 2. Quality of evidence assessment (results of the Cambridge Quality Checklist [40])

First author	Year	Cambridge Quality Checklist			Total Quality score (3–15)
		Checklist for correlates (1–5)	Checklist for risk factors (1–3)	Checklist for causal risk factor (1–7)	
Kavoussi [42]	2021	1	2	4	7
Fathi [43]	2021	2	3	4	9
Abbasi [44]	2020	1	2	3	6
Kamal [45]	2020	2	3	6	11
Omar [46]	2020	1	3	3	7
Mostafa [47]	2020	2	3	3	8
Camargo [48]	2019	1	3	3	7
Afsin [49]	2018	3	3	3	9
Vahidi [50]	2018	2	3	3	8
Sun [51]	2018	2	3	3	8
Zaazaa [53]	2018	2	3	7	12
Lu [52]	2018	2	3	3	8
Abdelbaki [54]	2017	2	3	4	9
Ni [55]	2016	3	3	4	10
Telli [56]	2015	3	3	3	9
Tavalaee [57]	2015	2	3	3	8
Mohammed [58]	2015	3	3	3	9
Smit [59]	2013	3	3	3	9
Li [60]	2012	1	3	3	7
Gabriel [61]	2012	3	3	3	9
La Vignera [62]	2012	3	3	3	9
Sadek [63]	2011	3	3	4	10
Zini [64]	2011	3	3	3	9
Ghazi [65]	2011	2	3	3	8
Dada [66]	2010	0	3	5	8
Nasr Esfahani [67]	2010	2	3	6	11
Smit [34]	2010	3	3	3	9
Zini [68]	2005	4	2	3	9
Yeşilli [69]	2005	1	3	4	8

1) Subgroup analysis: technique of varicocele repair

Subgroup analysis was performed based on the type of VR (including microsurgical, non-microsurgical inguinal, high retroperitoneal, laparoscopic, embolization, and others).

In 13 studies using microsurgical treatment, the random effect model (heterogeneity: I^2=80.517%) showed that VR significantly decreases SDF (SMD: –1.014; 95% CI: –1.263, –0.765; p<0.0001) (Fig. 3).

Sensitivity analysis was performed but no study was sensitive enough to change the conclusion that microsurgical repair of varicocele significantly decreases SDF (Supplement Fig. 3). Publication bias was apparent in those studies according to the funnel plot and Egger's test (p=0.028). By bias correction, the adjusted effect value indicated that SDF in varicocele men was robustly reduced after microsurgical repair (Supplement Fig. 4).

In four studies using the non-microsurgical inguinal method, the random effect model (heterogeneity: 90%–50%) showed that VR significantly decreases SDF (SMD: –1.495; 95% CI: –2.116, –0.873; p<0.0001) (Fig. 4).

2) Subgroup analysis: technique of sperm DNA fragmentation analysis

Additionally, subgroup analysis was checked according to the techniques used in SDF estimation (including sperm terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling [TUNEL],

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Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Kavoussi et al [42]	2021	−1.741	0.151	0.023	−2.037	−1.445	−11.532	0.000
	Fathi et al [43]	2021	−1.713	0.246	0.061	−2.196	−1.230	−6.950	0.000
	Abbasi et al [44]	2020	−0.375	0.304	0.093	−0.971	0.222	−1.231	0.218
	Camargo et al [48]	2019	−0.611	0.289	0.084	−1.178	−0.044	−2.112	0.035
	Afsin et al [49]	2018	−1.272	0.245	0.060	−1.752	−0.791	−5.188	0.000
	Vahidi et al [50]	2018	−1.077	0.276	0.076	−1.619	−0.536	−3.898	0.000
	Sun et al [51] (1)	2018	−1.484	0.123	0.015	−1.725	−1.243	−12.083	0.000
	Sun et al [51] (2)	2018	−1.453	0.121	0.015	−1.690	−1.216	−12.016	0.000
	Zaazaa et al [53]	2018	−1.345	0.248	0.061	−1.831	−0.860	−5.434	0.000
	Abdelbaki et al [54]	2017	−1.305	0.211	0.045	−1.764	−0.936	−6.389	0.000
	Ni et al [55] (1)	2016	−0.609	0.332	0.110	−1.260	0.041	−1.836	0.066
	Ni et al [55] (2)	2016	−0.750	0.345	0.119	−1.426	−0.074	−2.175	0.030
	Ni et al [55] (3)	2016	−1.140	0.407	0.166	−1.938	−0.341	−2.797	0.005
	Telli et al [56]	2015	−1.852	0.199	0.040	−2.243	−1.462	−9.297	0.000
	Tavalaee et al [57]	2015	−4.431	0.548	0.300	−5.505	−3.356	−8.085	0.000
	Mohammed et al [58]	2015	−2.073	0.202	0.041	−2.470	−1.676	−10.238	0.000
	Smit et al [59]	2013	−0.359	0.204	0.041	−0.758	0.040	−1.763	0.078
	Li et al [60]	2012	−0.419	0.328	0.108	−1.062	0.224	−1.278	0.201
	Gabriel et al [61]	2012	−1.040	0.403	0.162	−1.829	−0.251	−2.582	0.010
	La Vignera et al [62]	2012	−1.355	0.286	0.082	−1.916	−0.794	−4.733	0.000
	Sadek et al [63]	2011	−0.724	0.172	0.030	−1.061	−0.386	−4.206	0.000
	Zini et al [64] (1)	2011	−0.790	0.294	0.086	−1.366	−0.214	−2.690	0.007
	Zini et al [64] (2)	2011	−0.936	0.342	0.117	−1.606	−0.266	−2.740	0.006
	Zini et al [64] (3)	2011	−1.364	0.360	0.130	−2.070	−0.658	−3.788	0.000
	Ghazi and Abdelfattah [65]	2011	−0.662	0.160	0.026	−0.976	−0.347	−4.126	0.000
	Dada et al [66]	2010	−4.151	0.874	0.764	−5.865	−2.438	−4.748	0.000
	Nasr Esfahani et al [67]	2010	−4.054	0.295	0.087	−4.633	−3.475	−13.723	0.000
	Smit et al [34] (1)	2010	−0.462	0.257	0.066	−0.966	0.042	−1.795	0.073
	Smit et al [34] (2)	2010	−0.152	0.334	0.111	−0.807	0.502	−0.457	0.648
	Zini et al [68]	2005	−0.182	0.233	0.054	−0.639	0.275	−0.781	0.435
Fixed			**−1.243**	**0.042**	**0.002**	**−1.325**	**−1.161**	**−29.681**	**0.000**
Random			**−1.256**	**0.139**	**0.019**	**−1.529**	**−0.982**	**−9.013**	**0.000**

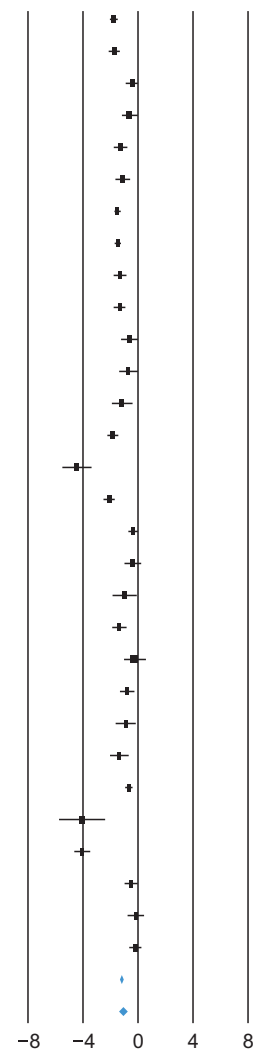

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Fig. 2. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair. 95% CI: 95% confidence interval.

sperm chromatin structure assay [SCSA], chromatin dispersion assay [SCD] and others).

In four studies using SCD to measure SDF, the random effect model (heterogeneity: I^2=94.958%), showed that VR significantly decreases the SDF (SMD: –2.197; 95% CI: –3.187, –1.207; p<0.0001) (Fig. 5). Sensitivity analysis was performed but no study was sensitive enough to change the main result (Supplement Fig. 5). Funnel plot and Egger's test (p=0.487) found that there was no publication bias in the four papers (Supplement Fig. 6).

In 6 studies using SCSA to analyze SDF, the random effect model (heterogeneity: I^2=81.446%) showed there was a significant decrease in SDF (SMD: –0.857; 95% CI: –1.156, –0.559; p<0.0001) (Fig. 6).

Sensitivity analysis was performed but did not reveal a change in the main meta-analysis result (Supplement Fig. 7). Publication bias was apparent in those studies according to the funnel plot and Egger's test (p=0.022). By bias adjustment, the corrected effect value indicated that SDF in varicocele men was robustly reduced after surgical repair when using SCSA testing (Supplement Fig. 8).

In five studies using TUNEL to measure SDF,

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Kavoussi et al [42]	2021	−1.741	0.151	0.023	−2.037	−1.445	−11.532	0.000
	Fathi et al [43]	2021	−1.713	0.246	0.061	−2.196	−1.230	−6.950	0.000
	Abbasi et al [44]	2020	−0.375	0.304	0.093	−0.971	0.222	−1.232	0.218
	Camargo et al [48]	2019	−0.611	0.289	0.084	−1.178	−0.044	−2.112	0.035
	Vahidi et al [50]	2018	−1.077	0.276	0.076	−1.619	−0.536	−3.898	0.000
	Sun et al [51] (1)	2018	−1.484	0.123	0.015	−1.725	−1.243	−12.083	0.000
	Sun et al [51] (2)	2018	−1.453	0.121	0.015	−1.690	−1.216	−12.016	0.000
	Zaazaa et al [53]	2018	−1.345	0.248	0.061	−1.831	−0.860	−5.434	0.000
	Ni et al [55] (1)	2016	−0.609	0.332	0.110	−1.260	0.041	−1.836	0.066
	Ni et al [55] (2)	2016	−0.750	0.345	0.119	−1.426	−0.074	−2.175	0.030
	Ni et al [55] (3)	2016	−1.140	0.407	0.166	−1.938	−0.341	−2.797	0.005
	Li et al [60]	2012	−0.419	0.328	0.108	−1.062	0.224	−1.278	0.201
	Gabriel et al [61]	2012	−1.040	0.403	0.162	−1.829	−0.251	−2.582	0.010
	La Vignera et al [62]	2012	−1.355	0.286	0.082	−1.916	−0.794	−4.733	0.000
	Zini et al [68]	2005	−0.182	0.233	0.054	−0.639	0.275	−0.781	0.435
	Zini et al [64]	2011	−0.790	0.294	0.086	−1.366	−0.214	−2.690	0.007
	Ghazi and Abdelfattah [65]	2011	−0.662	0.160	0.026	−0.976	−0.347	−4.126	0.000
Fixed			**−1.180**	**0.052**	**0.003**	**−1.283**	**−1.078**	**−22.622**	**0.000**
Random			**−1.014**	**0.127**	**0.016**	**−1.263**	**−0.765**	**−7.994**	**0.000**

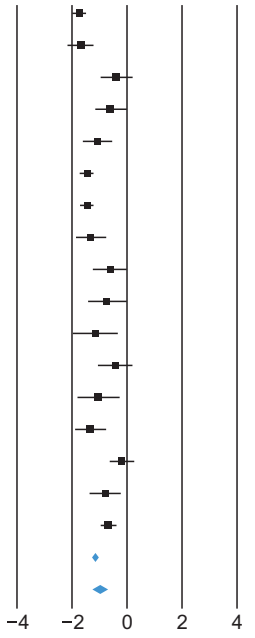

Fig. 3. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair using microsurgical treatment. 95% CI: 95% confidence interval.

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Sadek et al [63]	2011	−0.724	0.172	0.030	−1.061	−0.386	−4.206	0.000
	Telli et al [56]	2015	−1.852	0.199	0.040	−2.243	−1.462	−9.297	0.000
	Mohammed et al [58]	2015	−2.073	0.202	0.041	−2.470	−1.676	−10.238	0.000
	Abdelbaki et al [54]	2017	−1.350	0.211	0.045	−1.764	−0.936	−6.389	0.000
Fixed			**−1.436**	**0.097**	**0.009**	**−1.627**	**−1.246**	**−14.771**	**0.000**
Random			**−1.495**	**0.317**	**0.101**	**−2.116**	**−0.873**	**−4.714**	**0.000**

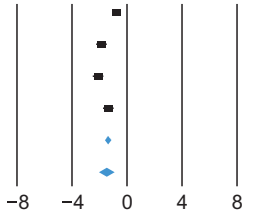

Fig. 4. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair using non-microsurgical inguinal treatment. 95% CI: 95% confidence interval.

the random effect model (heterogeneity: I^2=90.588%) showed that VR significantly decreases SDF (SMD: –1.599; 95% CI: –2.478, –0.719; p<0.0001) (Fig. 7). Sensitivity analysis was performed but no study was sensitive enough to change the conclusion (Supplement Fig. 9). The funnel plot and Egger's test (p=0.116) found that there was no publication bias in the five papers (Supplement Fig. 10).

In the three studies which did not describe the specific testing methods for SDF, the random effect model (heterogeneity: I^2=95.361%) showed that VR significantly improved the SDF (SMD: –1.375; 95% CI: –2.483, –0.266; p=0.015) (Fig. 8). Sensitivity analysis was performed but no study was sensitive enough to change the conclusion (Supplement Fig. 11). The funnel plot and Egger's test (p=0.137) found that there was no publication bias in the three papers (Supplement Fig. 12).

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Kavoussi et al [42]	2021	−1.741	0.151	0.023	−2.037	−1.445	−11.532	0.000
	Fathi et al [43]	2021	−1.713	0.246	0.061	−2.196	−1.230	−6.950	0.000
	Zaazaa et al [53]	2018	−1.345	0.248	0.061	−1.831	−0.860	−5.434	0.000
	Nasr Esfahani et al [67]	2010	−4.054	0.295	0.087	−4.633	−3.475	−13.723	0.000
Fixed			**−1.963**	**0.107**	**0.011**	**−2.127**	**−1.755**	**−18.429**	**0.000**
Random			**−2.197**	**0.505**	**0.255**	**−3.187**	**−1.207**	**−4.350**	**0.000**

Std diff in means and 95% CI

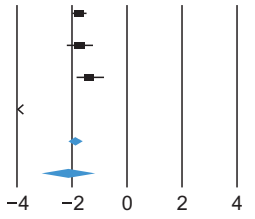

Fig. 5. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair using SCD. 95% CI: 95% confidence interval.

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Sun et al [51] (1)	2018	−1.484	0.123	0.015	−1.725	−1.243	−12.083	0.000
	Sun et al [51] (2)	2018	−1.453	0.121	0.015	−1.690	−1.216	−12.016	0.000
	Abdelbaki et al [54]	2017	−1.350	0.211	0.045	−1.764	−0.936	−6.389	0.000
	Ni et al [55] (1)	2016	−0.609	0.332	0.110	−1.260	0.041	−1.836	0.066
	Ni et al [55] (2)	2016	−0.750	0.345	0.119	−1.426	−0.074	−2.175	0.030
	Ni et al [55] (3)	2016	−1.140	0.407	0.166	−1.938	−0.341	−2.797	0.005
	Smit et al [59]	2013	−0.359	0.204	0.041	−0.758	−0.040	−1.763	0.078
	Li et al [60]	2012	−0.419	0.328	0.108	−1.062	0.224	−1.278	0.201
	Gabriel et al [61]	2012	−1.040	0.403	0.162	−1.829	−0.251	−2.582	0.010
	Sadek et al [63]	2011	−0.724	0.172	0.030	−1.061	−0.386	−4.206	0.000
	Smit et al [34] (1)	2010	−0.462	0.257	0.066	−0.966	0.042	−1.795	0.073
	Smit et al [34] (2)	2010	−0.152	0.334	0.111	−0.807	0.502	−0.457	0.648
Fixed			**−1.073**	**0.060**	**0.004**	**−1.191**	**−0.956**	**−17.898**	**0.000**
Random			**−0.857**	**0.152**	**0.023**	**−1.156**	**−0.559**	**−5.627**	**0.000**

Std diff in means and 95% CI

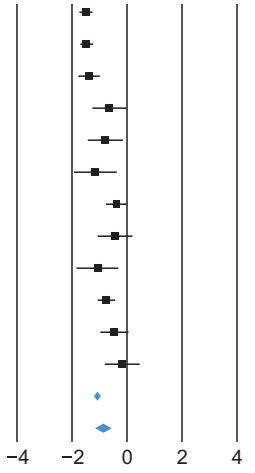

Fig. 6. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair using SCSA. 95% CI: 95% confidence interval.

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	La Vignera et al [62]	2012	−1.355	0.286	0.082	−1.916	−0.794	−4.733	0.000
	Tavalaee et al [57]	2015	−4.431	0.548	0.300	−5.505	−3.356	−8.085	0.000
	Afsin et al [49]	2018	−1.272	0.245	0.060	−1.752	−0.791	−5.188	0.000
	Vahidi et al [50]	2018	−1.077	0.276	0.076	−1.619	−0.536	−3.898	0.000
	Abbasi et al [44]	2020	−0.375	0.304	0.093	−0.971	0.222	−1.232	0.218
Fixed			**−1.259**	**0.134**	**0.018**	**−1.521**	**−0.997**	**−9.428**	**0.000**
Random			**−1.599**	**0.449**	**0.202**	**−2.478**	**−0.719**	**−3.561**	**0.000**

Std diff in means and 95% CI

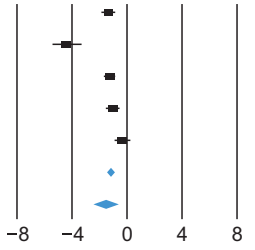

Fig. 7. Forest plot of the sperm DNA fragmentation in infertile patients after varicocele repair compared to before varicocele repair using TUNEL. 95% CI: 95% confidence interval.

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Telli et al [56]	2015	−1.852	0.199	0.040	−2.243	−1.462	−9.297	0.000
	Mohammed et al [58]	2015	−2.073	0.202	0.041	−2.470	−1.676	−10.238	0.000
	Zini et al [68]	2005	−0.182	0.233	0.054	−0.639	0.275	−0.781	0.435
Fixed			**−1.479**	**0.121**	**0.015**	**−1.717**	**−1.241**	**−12.197**	**0.000**
Random			**−1.375**	**0.566**	**0.320**	**−2.483**	**−0.266**	**−2.430**	**0.015**

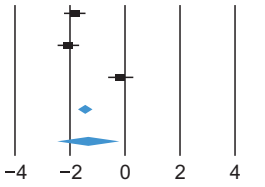

Fig. 8. Forest plot of the sperm DNA fragmentation (SDF) in infertile patients after varicocele repair compared to before varicocele repair using a not specified methods for analysis of SDF. 95% CI: 95% confidence interval.

Model	Study name	Year	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-value	p-value
	Kamal et al [45]	2020	−1.687	0.282	0.080	−2.240	−1.133	−5.973	0.000
	Omar et al [46]	2020	−6.208	0.341	0.116	−6.876	−5.539	−18.200	0.000
	Mostafa et al [47]	2020	−1.437	0.290	0.084	−2.004	−0.869	−4.961	0.000
	Lu et al [52]	2018	−7.256	0.749	0.562	−8.725	−5.787	−9.683	0.000
	Ni et al [55] (1)	2016	−0.868	0.339	0.115	−1.533	−0.203	−2.557	0.011
	Ni et al [55] (2)	2016	−1.020	0.354	0.126	−1.715	−0.326	−2.879	0.004
	Ni et al [55] (3)	2016	−1.194	0.410	0.168	−1.998	−0.390	−2.911	0.004
	Yeşilli et al [69]	2005	−0.392	0.280	0.078	−0.941	0.157	−1.401	0.161
Fixed			**−1.885**	**0.119**	**0.014**	**−2.119**	**−1.651**	**−15.794**	**0.000**
Random			**−2.450**	**0.741**	**0.549**	**−3.903**	**−0.997**	**−3.306**	**0.001**

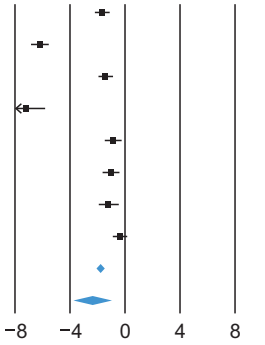

Fig. 9. Forest plot of seminal malondialdehyde level in infertile patients after varicocele repair compared to before varicocele repair. 95% CI: 95% confidence interval.

2. Malondialdehyde

A total of 6 studies analyzed seminal MDA as an outcome before and after VR in infertile men. The random effect model (heterogeneity: I^2=97.3%) showed that VR significantly reduced seminal MDA levels (SMD: –2.450; 95% CI: –3.903, –0.997; p=0.001; MD: –2.394; 95% CI –2.524, –1.275; p<0.001) (Fig. 9). As an overall, there appeared to be no study that was sensitive enough to alter the findings that VR lowers seminal MDA levels (Supplement Fig. 13). Based on the symmetry of the standard error and precision Funnel plots respectively (Supplement Fig. 14), as well as the Egger's test (p>0.05), no publication bias was apparent.

3. Meta-regression analysis

The meta-regression analysis revealed that the SDF reduction after VR was independent of the preoperative SDF levels. In the present analysis, the magnitude of the effect size did not increase as a function of preoperative SDF levels (coefficient: –0.01; 95% CI: –0.03, 0.02; p=0.64) (Fig. 10).

DISCUSSION

Varicocele is linked with DNA damage of spermatozoa due to elevated OS and decreased antioxidant capacity. In this meta-analysis, we strove to evaluate the effect of VR on SDF and seminal MDA levels in infertile patients with clinical varicocele. For SDF, a total of 23 studies were found to investigate the SDF differences before and after VR and a significant reduction was seen in SDF levels after VR. The improvement in SDF levels after VR emerged regardless of the surgical technique and methods that were used in SDF estimation. For the MDA, a total of six studies were included in the analysis, finding a significant decrease after VR.

The World Journal of MEN's HEALTH

The American Urological Association/American Society for Reproductive Medicine (AUA/ASRM) guideline on male infertility suggests performing VR in the setting of clinically palpable varicocele, infertility, and abnormal sperm parameters on semen analysis [70]. This guideline does not specifically address the role of VR in men with abnormal SDF. The European Association of Urology (EAU) male infertility guideline states that varicoceles can be a source of elevated SDF, and VR can be considered in men with increased SDF with abnormal semen parameters [71]. It is unclear whether VR should be offered to men who have elevated SDF in the setting of normal semen parameters, unexplained infertility, or those who have suffered from failed ART or RPL [71].

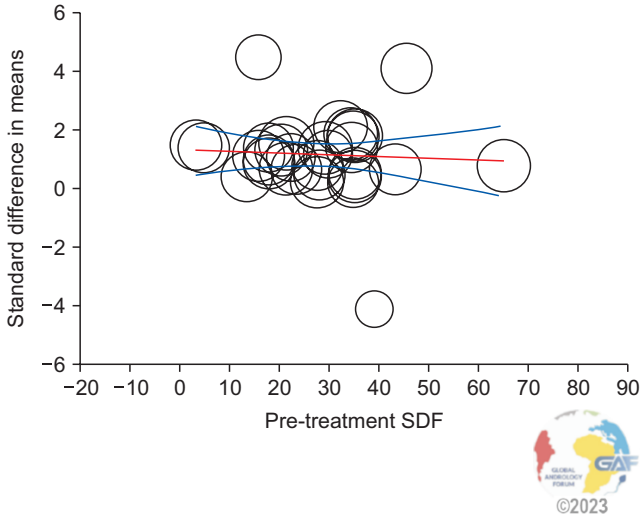

Fig. 10. Metaregression showing the influence of pre-treatment sperm DNA fragmentation (SDF) levels (moderator) on the standard difference of the mean of SDF after varicocelectomy. The size of the circles indicates the studies'sample sizes. The red line represents linear predictions for the standard mean difference of SDF change after varicocelectomy as a function of the mean absolute increase in preoperative SDF levels. The curved lines indicate the 95% confidence interval lines around the regression line.

The lack of specific guidelines concerning the benefits and indications of VR for male infertility may raise doubts as to the implementation of this therapy in daily clinical practice [71]. Several studies have shown that there was an increase in SDF in varicocele cases and that VR could reduce these SDF levels [34]. Furthermore, increased levels of MDA can also be used as an indicator of high OS which is also one of the proposed effects of varicocele. Additionally, significant improvement was seen in MDA levels after VR, indicating a reduction in OS.

Of the 6 studies examined in the SRMA on MDA, all were found to show a significant reduction after VR. Previous studies have shown that MDA levels are increased in men with varicocele, and it has been shown that VR reduces MDA levels [45]. When calculated cumulatively with this SRMA, the increase in MDA levels in varicocele patients and the decrease after VR support the concept that VR decreases the levels of sperm lipoperoxidation, which can concur with loss of pregnancy [24].

We examined the strengths of our MA compared to previously published SRMA (Table 3 and Fig. 11).

Table 3. Comparison with previous meta-analyses

Study (publication year)	No. of studies/ patients (total)	Study designs included in SRMA	The measure of sperm DNA damage	Outcomes
Present study	29/1,523 6/268[a]	Prospective, retrospective, and unspecified	SCSA, SCD, TUNEL, and others 8OHDG: Didn't qualify for meta-analysis Comet: Didn't qualify for meta-analysis	Varicocele repair reduces sperm DNA damage and MDA levels.
Lira Neto et al (2021) [76]	19/1,070	Prospective and retrospective	SCSA, TUNEL, SCD, Comet	Varicocele repair reduces sperm DNA damage.
Birowo et al (2020) [75]	7/289	Prospective	SCSA, TUNEL	Varicocele repair reduces sperm DNA damage.
Qiu et al (2021) [73]	11/394	Prospective	SCSA, TUNEL, SCD, Comet, AOT	Varicocele repair reduces sperm DNA damage.
Wang et al (2012) [74]	6/177	Prospective, retrospective, and unspecified	SCSA, TUNEL, Comet	Varicocele repair reduces sperm DNA damage.
Baazeem et al (2011) [72]	3/84	Prospective	SCSA	Varicocele repair reduces sperm DNA damage.

SRMA: systematic review and meta-analysis, SCSA: sperm chromatin structure assay, SCD: Sperm Chromatin Dispersion, TUNEL: terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling, 8OHDG: 8-hydroxydeoxyguanosine, AOT: acridine orange test.
[a]This number represents MDA studies only.

Baazeem et al [72] in their meta-analysis took into account only 3 prospective studies about sperm DNA damage. In those studies, all the investigators recorded pre- and post-VR sperm DFI (DNA fragmentation index). Two out of 3 studies found a statistically significant decrease in DFI postoperatively, however, in the remaining study, only 10 out of 25 patients completed the follow-up so the improvement in DNA fragmentation was not significant. In our opinion, the strength of this meta-analysis is that all the articles involved used the same method (SCSA) to measure the DFI for better comparison. Another recent meta-analysis involved 11 prospective studies with various methods of DNA damage measurement [73]. In the present meta-analysis, patients served as their controls, as preoperative parameters were used as control values. On average, the DFI % of clinical varicocele patients decreased by 5.79 (95% CI, −7.39 to−4.19) after VR. Furthermore, in the fixed effect model for 10 studies (number of participants=364, heterogeneity chi-squared=15.15, df=9, p=0.087, I^2=40.6%), DFI% decreased by 6.14 (95% CI, −6.90 to −5.37) on average.

In their 2012 MA, Wang and colleagues reported that patients with varicoceles had significantly higher sperm DNA damage than controls, with a MD of 9.84% (95% CI 9.19–10.49; p<0.00001). Authors stated that a VR performed with either microsurgical or an unspecified method can improve sperm DNA integrity, with a MD of 3.37% (95% CI –4.09 to –2.65; p<0.00001) [74].

In another recent meta-analysis [75], the sperm DFI decreased by 6.86% after VR (MD: –6.86; 95% CI: −10.04, −3.69; p<0.00001). SCSA was the preferred method in most of the included studies, with the exception of two studies that used the TUNEL assay.

The most recent and largest MA on this topic was reported by Lira Neto et al [76] in 2021. VR was associated with postoperative decreased SDF rates (weighted mean difference –7.23%; 95% CI: –8.86 to –5.59; I^2=91%). The pooled results were consistent for studies using SCSA, TUNEL, SCD, Comet, and microsurgical VR which in our opinion is a strength of this SRMA. Subgroup analyses in the latter study demonstrated a more pronounced treatment effect in men with elevated preoperative SDF levels. Indeed, their meta-regression analysis showed that SDF decreased after VR as a function of preoperative SDF levels (coefficient: 0.23; 95% CI 0.07–0.39). Surprisingly, the present study found that SDF reduction after VR was independent of the preoperative SDF levels. In other words, this indicates that the extent of SDF improvement after VR is similar in both patients with either high and low SDF. The reasons for the finding of opposing results are not immediately clear. The methods used in this study are very similar to those used in the study by Lira Neto et al [76]. However, having been conducted more recently, it could be supposed that the increase in the size of the population examined in the present study (which is the largest examined so far) could represent one of the main factors responsible for the results.

Till now, five meta-analyses were conducted with a total of 2,014 patients investigating the effect of VR on SDF. Four meta-analyses included prospective studies and one meta-analysis included both prospective and retrospective studies. All meta-analyses indicate that sperm DNA damage is reduced after VR. Our current meta-analysis carried out on 1,523 patients (considering only studies on SDF), confirms and supports the current literature, adding 453 patients.

Up until now, there was no meta-analysis in the literature investigating the effect of VR on seminal levels of MDA in infertile patients with clinical varicocele. An interesting aspect of our meta-analysis is that it reports, for the first time, a reduction in MDA after VR.

Before concluding we would like to underline the strength and limitations of this study using a SWOT (strengths, weaknesses, opportunities, and threats) analysis (Fig. 11). The strengths of the present study were the inclusion of 29 articles that collected a total of 1491 patients. Additionally, we conducted subgroup analyses to assess heterogeneity and conducted the sensitivity analysis. Finally, our SRMA is the first one to have meta-analyzed the effects of VR on MDA. One limitation was the fact that our meta-analysis did not compare the impact of one VR technique to another. Furthermore, we did not evaluate the impact of different degrees of varicocele on SDF improvement after VR. Finally, we were unable to assess the impact of VR on the SDF rate as evaluated by the 8-hydroxydeoxyguanosine or Comet assays. The results of this SRMA should be a guide for professional organizations connected with the field of male infertility to further guide on the need for VR in men with not only quantitative indicators of male infertility in the conventional semen analysis but qualitative indicators of sperm dysfunction via SDF and MDA.

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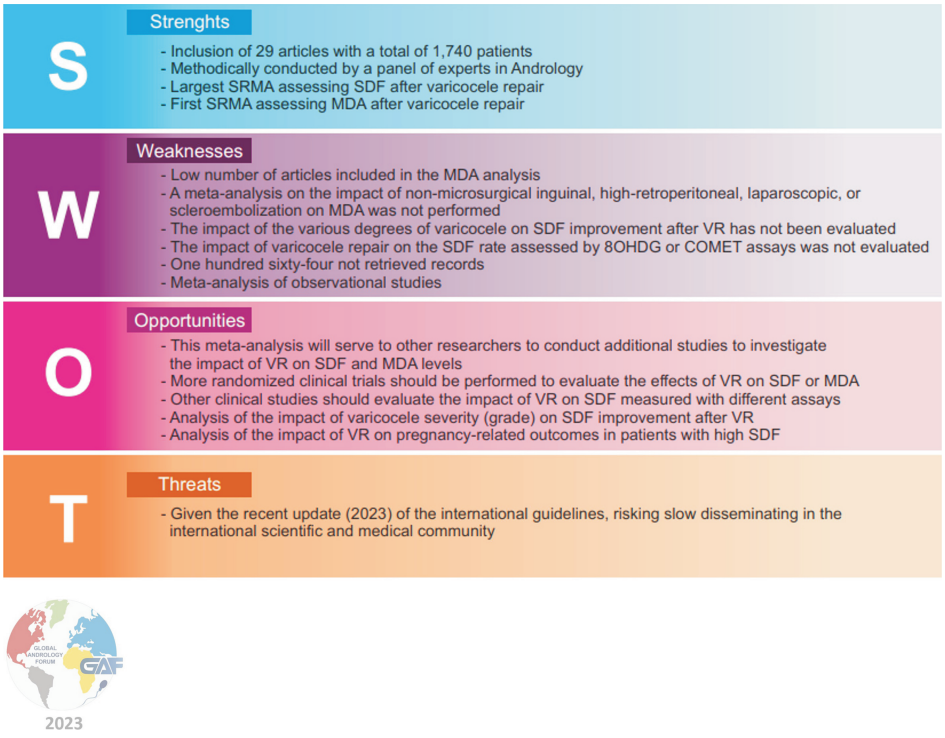

Fig. 11. Strengths, Weaknesses, Opportunities, and Threats (SWOT) Analysis. SRMA: systematic review and meta-analysis, SDF: sperm DNA fragmentation, MDA: malondialdehyde, VR: varicocele repair, 8OHDG: 8-hydroxydeoxyguanosine.

CONCLUSIONS

By analyzing the largest sample so far, this SRMA provides additional evidence that VR significantly improves SDF. Reductions of SDF were observed independent of the surgical technique, type of SDF testing assay, or preoperative SDF values. To the best of our knowledge, the present study is the first SRMA evaluating the effect of VR on seminal MDA levels and demonstrating significant decrease of this OS biomarker following repair. Our findings further strengthen currently available evidence and could potentially serve to upgrade the professional societies' practice recommendations favoring VR to improve SDF and to ameliorate seminal OS in infertile men.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: RC, AA, R Shah. Data curation: R Singh, PS. Formal analysis: R Singh, PS. Investigation: RC, R Shah. Methodology: RC, GS, F Boitrelle. Project administration: AA, R Shah. Software: R Singh. Visualization: all authors. Writing – original draft: all authors. Writing – review & editing: all authors.

Supplementary Materials

Supplementary materials can be found via https://doi.org/10.5534/wjmh.230235.

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