MALE INFERTILITY: THE FUNCTIONAL SIDE OF THE SPERMATOZOA

Ph.D. Thesis

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1. Introduction

1.1. Overview of the topic

1.1.1. What is the topic?

The topic of this thesis is the role of SDF in male infertility, currently the only evidence-based sperm functional parameter incorporated into clinical guidelines. This work focuses on identifying risk factors associated with elevated SDF and evaluating interventions aimed at reducing SDF levels. The research is based on two comprehensive meta-analyses – one examining the contributing risk factors and the other assessing the effectiveness of various therapeutic strategies to lower SDF.

1.1.2. What is the problem to solve?

Infertility affects approximately 15% of couples globally, with male factors implicated in more than half of these cases. Traditional semen parameters often fail to identify underlying causes. SDF has emerged as a functional biomarker with strong predictive value for fertility outcomes, yet the exact

risk factors contributing to increased SDF, and the effectiveness of treatments to reduce it, remain unclear. Other limitations can also be mentioned, as there is no gold standard laboratory method for measurement, nor a universal threshold to differentiate fertile from infertile men based on SDF. Thus, better understanding both the risk factors and therapeutic options for high SDF is crucial for targeted clinical management.

1.1.3. What is the importance of the topic?

SDF is associated with decreased fertility, lower success rates in assisted reproductive techniques, increased miscarriage rates and higher foetal abnormalities. By identifying risk factors such as varicocele, smoking, pollution, age, and impaired glucose tolerance, and by evaluating treatments like varicocelectomy, antioxidant therapy, FSH administration, and lifestyle modifications, this research provides critical insights for personalized fertility care. Addressing SDF may improve

reproductive outcomes, and guide future guideline recommendations.

1.1.4. What would be the impact of our research results?

The results of our research have the potential to significantly influence both clinical practice and future scientific work. By identifying the most relevant risk factors contributing to elevated sperm DNA fragmentation and assessing the efficacy of various interventions aimed at reducing it, our findings offer valuable guidance for the individualized management of male infertility. Clinicians will be better equipped to make evidence-based decisions regarding which patients may benefit from specific treatments, such as varicocelectomy or FSH therapy. Additionally, our work highlights the limitations of antioxidant therapy and the need for more robust studies on lifestyle interventions. Importantly, our research also draws attention to the current lack of

standardization in measuring and evaluating SDF, highlighting the necessity of establishing reliable, reproducible diagnostic protocols.

1.2. Sperm DNA fragmentation assays

Several assays have been developed to evaluate SDF, each with distinct methodologies and diagnostic characteristics. The Sperm Chromatin Structure Assay (SCSA) is a flow cytometry-based method that detects DNA denaturation using acridine orange staining and calculates the DNA fragmentation index (DFI) based on fluorescence ratios. It offers high reproducibility, large detection capacity, and low mutation rates, although it assesses DNA susceptibility rather than direct strand breaks.

The Comet assay, or single-cell gel electrophoresis, directly visualizes DNA strand breaks as a "comet tail" formed during electrophoresis under alkaline or neutral conditions. It is relatively cheap, sensitive,

and adaptable, though results are dependent on operating conditions and detection thresholds.

Terminal deoxynucleotidyl transferase deoxynucleotidyl transferase (dUTP) nick end labelling (TUNEL) is another direct assay that labels DNA strand breaks with fluorescent markers and is considered highly accurate. However, it requires careful handling and has limited sensitivity under microscopy.

The Sperm Chromatin Dispersion (SCD) test evaluates DNA integrity by visualizing halo formation around sperm nuclei under fluorescence microscopy. This method is affordable, and easy to perform, though it indirectly assesses DNA damage.

While these assays are largely comparable in identifying elevated SDF, they vary in sensitivity, specificity, and what aspect of DNA damage they assess.

2. Objectives

2.1. Study I. – Investigating the effect of risk factors on SDF

Our goal was to assess the effect of all studied risk factors on SDF.

2.2. Study II. – Investigating the effect of interventions on SDF

Our aim was to summarise the effects of all interventions studied in relation to SDF.

3. Methods

The systematic reviews and meta-analyses were reported based on the recommendation of the PRISMA 2020 guideline, and we followed the Cochrane Handbook. Furthermore, the study protocols were registered on PROSPERO with the registration numbers CRD42021282533 (Study I) and CRD42021283784 (Study II).

3.1. Study I. – Investigating the effect of risk factors on SDF

A search was conducted in MEDLINE (via PubMed), Embase, and CENTRAL from inception to October 17, 2021, without language restrictions. Eligible studies included prospective or retrospective cohort studies comparing SDF values between groups with and without predefined risk factors, such as lifestyle, environmental, or health-related exposures. All major SDF assays (e.g., SCSA, TUNEL, SCD, Comet) were accepted. Outcomes were reported as mean differences (MDs) with

standard deviations (SD) or odds ratios (ORs) with 95% confidence intervals (CI). Data were pooled using a random-effects model, and heterogeneity was assessed via I^2 and Cochrane's Q tests. Subgroup analyses were conducted based on fertility status and assay types. Risk of bias was assessed

using the QUIPS tool. Statistical analyses were performed in R using the *meta* and *dmetar* packages.

3.2. Study II. – Investigating the effect of interventions on SDF

The previous search was updated on January 3, 2023. Eligible studies included randomized controlled trials, prospective or retrospective cohorts assessing interventions intended to improve SDF in men of any fertility status. Interventions included mainly varicocelectomy, lifestyle changes, hormonal therapy, and antioxidant supplementation. Comparators were either untreated controls or pre-intervention data. Any SDF assay was accepted (e.g., SCSA, TUNEL, Comet, SCD), and data were synthesized as MDs with SDs.

Risk of bias was assessed using MINORS, ROBINS-I, or RoB2 tools, and quality of evidence for RCTs was evaluated using the GRADEpro tool. Statistical analyses were performed in R using random-effects models with Hartung-Knapp

adjustment. Heterogeneity was assessed via the I^2 statistic and Cochrane's Q tests, and publication bias was examined using funnel plots and Egger's test for outcomes with sufficient studies. Sensitivity analyses were conducted using leave-one-out methods. Standard deviation of change was imputed using a correlation of 0.6 based on Cochrane guidance.

4. Results

4.1. Study I. – Investigating the effect of risk factors on SDF

A total of 190 studies were included from an initial pool of 26,901 records. Most studies were retrospective and examined male patients in their 30s attending fertility clinics. The most frequently examined risk factor was varicocele, which was associated with a clinically relevant increase in SDF (MD = 13.62%, CI: 9.39–17.84), particularly when

palpable. Other health-related conditions such as impaired glucose tolerance (MD = 13.75%, CI: 6.99–20.51) and testicular tumors (MD = 11.30%, CI: 7.84–14.76) also showed notable SDF elevation. Among lifestyle factors, smoking had a dose-dependent effect, with heavy smoking significantly increasing SDF (MD = 9.60%, CI: 3.80–15.40), while alcohol use and BMI had no statistically significant impact. Age above 50 was associated with a marked rise in SDF (MD = 12.58%, CI: 7.31–17.86). Pollutant exposure (MD = 9.68%, CI: 6.85–12.52), ROS elevation (MD = 6.10%, CI: 4.65–7.55), spinal cord injury (up to MD = 60.8%), and heroin use (MD = 31.79%) were among the strongest contributors as well. Egger's test for publication bias showed no significant small-study effects for varicocele or age. However, heterogeneity was considerable across most risk factors due to varied definitions and populations.

4.2. Study II. – Investigating the effect of interventions on SDF

Eighty-six studies were selected from 36,531 articles, with most of them including infertile male populations. Varicocelectomy was assessed in 27 studies (1,818 men), showing the most pronounced SDF reduction at 6 months post-surgery (MD = –12.39%, CI: –22.41 to –2.36). Greater improvements were observed in grade III varicocele (MD = –7.35%, CI: –9.28 to –5.43) than grade II (MD = –4.55%, CI: –5.87 to –3.22). Antioxidant treatment was examined in 39 studies (4,958 men), with clinically not relevant SDF changes regardless of subgroups. At 3 months any monotherapy (MD = –3.36%, CI: –4.44 to –2.28) or any combined therapy (MD = –4.51%, CI: –6.81 to –2.20) resulted in only modest improvements. FSH treatment in 8 studies (637 men) resulted in a mean SDF reduction of –6.66% (CI: –9.64 to –3.69) at 3 months. Seven studies on lifestyle changes (587 men), mainly

involving exercise, showed a mild SDF decrease (MD = –2.94%, CI: –4.94 to –0.95). The greatest improvement among less common interventions was seen with antibiotics and anti-inflammatory treatment for infections (MD = –13.45%). Risk of bias was low for most RCTs and single-arm studies, though confounding reduced the quality in two-arm studies. GRADE assessment rated evidence certainty as low for varicocelectomy and very low for other interventions. Heterogeneity was high across all analyses due to population differences, assay variability, and measurement reproducibility issues.

5. Conclusions

Our studies identified several factors associated with increased SDF, including varicocele, impaired glucose tolerance, testicular tumors, smoking, environmental pollution, and advanced paternal age. These findings highlight the need to assess

underlying health and lifestyle factors in the evaluation of male fertility. In terms of management, we evaluated four major interventions for reducing SDF. Varicocelectomy – when correctly indicated – showed the most consistent and clinically meaningful improvement, particularly six months postoperatively. FSH therapy demonstrated moderate efficacy, while antioxidant supplementation and lifestyle modifications produced limited and inconsistent results. Together, these findings offer evidence-based guidance for a more individualized and targeted approach to the diagnosis and treatment of elevated SDF in men presenting with infertility.

6. Bibliography

-Publications related to the thesis:

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