

# Sperm DNA fragmentation testing in patients with subclinical varicocele: is there any evidence?

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The commentary by Drs. Herati and Lamb towards the clinical utility of sperm DNA fragmentation (SDF) testing article by Agarwal *et al.* (1) offers valuable views enriching the debate on this subject. The authors recognized the drawbacks of the conventional semen analysis results in accurately predicting the true male fertility potential, and acknowledged the value that sperm function tests, like SDF, might offer in this regards. As for its applicability in varicocele patients, the authors went further proposing the utilization of SDF in patients with subclinical varicocele to help in selecting men who will ultimately benefit from varicocelectomy. Finally, they highlighted the recommendations of the American Society for Reproductive Medicine (ASRM) (2) suggesting that due to “...*SDF testing’s low predictive ability of pregnancy outcomes, lack of validation between studies, and overall insufficient evidence correlating sperm DNA integrity and reproductive outcomes.*”, caution should be practiced before utilizing this test for assisted reproductive treatments (ART).

The medical literature includes few clinical conditions that despite being extensively researched remain entrenched in controversies. Varicocele is one good example where its primary disagreement is in selecting the right patient who may benefit from varicocelectomy. In fact, varicocele treatment is not always helpful in improving sperm production in every patient with this condition.

Studies on semen parameters and pregnancy outcomes of varicocelectomy have overall reported significantly better results among patients with clinical varicocele rather

than subclinical varicocele (3-5). This observation has led major professional societies to recommend treatment for varicocele only in patients with palpable disease (6). The grading of varicocele needs a high level of expertise which is usually obtained with advanced training or extensive exposure to male infertility cases. Three grades exist; grade 1 is considered when a filling sensation is felt along the spermatic veins as the patient performs the Valsalva maneuver; grade 2 is when the spermatic cord is felt like a bag of worms or a squishy tube; and grade 3 is the presence of visibly dilated and tortuous veins along the spermatic cord. It is generally difficult to accurately differentiate between subclinical varicocele and lower grades of clinical varicocele. As such duplex ultrasound examination of the testes has become an ideal method for assessing the diameter of the internal spermatic veins and the presence of reflux during Valsalva maneuver. A diameter of 3 mm has been recognized as the limit above which a varicocele could become palpable (7).

The authors have suggested the utilization of SDF for patients with subclinical varicocele as it might help in selecting those who would benefit from varicocelectomy. They cited Cantoro *et al.* (8), who performed a prospective evaluation of 337 men with subclinical varicoceles who were either managed by percutaneous embolization (n=218) or received no treatment (n=119). Embolization of the subclinical varicoceles in this treatment group resulted in an improved sperm concentration (16.5±4.6 million/mL at baseline; 37.4±10.7 million/mL after 6 months; P<0.05),

sperm motility ( $32.4\% \pm 10.6\%$  at baseline;  $46.6\% \pm 9.4\%$  after 6 months;  $P < 0.05$ ) and FSH ( $1.3 \pm 3.4$  mIU/mL at baseline;  $4.8 \pm 3.7$  mIU/mL after 6 months;  $P < 0.05$ ) values. More importantly, these authors reported a statistically significant improvement in clinical pregnancy rates among the treatment group (46.3%) in comparison with the no treatment group (11.8%) ( $P = 0.01$ ).

Fewer studies have investigated the outcome of treatment in patients with subclinical varicocele. A recent systemic review and meta-analysis by Kim *et al.* (5) evaluated seven randomized controlled trials with a total of 548 patients with subclinical varicocele comparing surgery to no-treatment or clomiphene citrate. Varicocelectomy was performed for 276 patients, while the remaining 272 patients either received no treatment or clomiphene citrate. The authors detected that varicocelectomy resulted in statistically significant improvements on forward progressive sperm motility (mean difference 3.94%, 95% CI: 1.24–6.65), however no statistically significant difference in pregnancy rate (OR 1.29, 95% CI: 0.99–1.67) was reported.

Contrary to the above-mentioned study, García-Peiró *et al.* (4) analyzed SDF levels using multiple analysis methods in semen samples from 60 infertile patients with varicocele. Patients were grouped into four groups: non-treated clinical grade 1 varicocele ( $n = 15$ ), surgically treated clinical varicocele ( $n = 19$ ), non-treated subclinical varicoceles ( $n = 16$ ), and surgically treated subclinical varicoceles ( $n = 10$ ). In comparison to the normal fertile group, substantial SDF levels were detected in clinical and subclinical varicocele patients. However, statistically significant reductions in SDF measures were only detected in patients with clinical varicocele when compared with subclinical varicocele groups ( $P < 0.05$ ).

While exploring the impact of varicocele ligation on the male fertility status, a recent study by Ni *et al.* (3) offers valuable insights into this debate. These authors investigated semen parameters, malondialdehyde (MDA) levels (a measure of lipid peroxidation) and SDF (using the SCSA method) in infertile patients with subclinical varicocele ( $n = 15$ ), normozoospermic patients with clinical varicocele ( $n = 22$ ) and infertile astheno/oligozoospermic patients with clinical varicocele ( $n = 51$ ) comparing the results to a control fertile group ( $n = 25$ ). While these authors reported significantly lower semen parameters in the subclinical varicocele group than the control group ( $P < 0.01$ ), no significant differences occurred in MDA and SDF levels between both groups ( $P > 0.05$ ). Based on current clinical practice guidelines, the same authors

performed microsurgical varicocelectomy in the infertile astheno/oligozoospermic patient group. To explore the effect of surgery and the influence of varicocele on sperm quality of untreated men, they performed the same semen studies in all the study groups 6 months after the initial results. No significant differences were reported in semen parameters, MDA and SDF in the subclinical varicocele group 6 months from the initial semen studies. Significant worsening in semen parameters, MDA and SDF levels was detected 6 months from the initial results in the normozoospermic clinical varicocele group who did not undergo surgery ( $P < 0.05$ ). Lastly, significant improvement in semen parameters, MDA and SDF levels were reported in the astheno/oligozoospermic clinical varicocele group 6 months after performing surgery ( $P < 0.05$ ). Regarding pregnancy outcome, these authors reported a pregnancy rate of 26.67% in the subclinical varicocele group, 27.27% in the normozoospermic varicocele group and 23.53% in the astheno/oligozoospermic varicocele group ( $\chi^2$  test,  $P > 0.05$ ). Importantly, the group of patients who successfully conceived postoperatively had a significantly lower SDF levels than those who did not conceive ( $22.28\% \pm 5.93\%$  vs.  $25.96\% \pm 5.98\%$ ,  $P < 0.05$ ). After performing receiver operator curve analysis of SDF levels to discriminate pregnant and non-pregnant couples, Ni *et al.* reported a SDF cutoff value of 19.73% to be associated with a sensitivity of 77.27% and a specificity of 72.73% (area under the curve 0.76).

As such, given the existing data examining the significance of SDF tests in patients with subclinical varicocele and the uncertainty regarding the clinical benefit of varicocelectomy in this group of patients, the utility of SDF tests for this patient population appears to be unjustified at this time (9). We have proposed the utilization of SDF in patients with grade 1 varicocele with borderline/abnormal semen analysis as recent studies have confirmed that surgery is associated with a significant reduction in SDF in this patient population (10,11) and more importantly the improvements in semen parameters and pregnancy rates witnessed after surgery were equivalent between low grade and high-grade varicocele (12).

Lastly, while the ASRM recommended against the routine use of SDF testing for the evaluation of infertile men, they did acknowledge that, especially in ART, the results offered by the SDF test may be valuable in the clinical setting (2). Several good quality studies (13–15) have been published recently solidifying the influence of SDF test results on the outcomes of ART suggesting that the time has come for updated recommendations by fertility

societies (reviewed by Agarwal *et al.*) (16).

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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