

# Sperm DNA fragmentation

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Comment on: Agarwal A, Majzoub A, Esteves SC, *et al.* Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* 2016;5:935-50.

Submitted Jan 25, 2017. Accepted for publication Apr 24, 2017.

doi: 10.21037/tau.2017.05.07

View this article at: <http://dx.doi.org/10.21037/tau.2017.05.07>

I agree with Agarwal *et al.* (1) that sperm DNA fragmentation (SDF) should be included in the evaluation of male factor infertility, along with semen analysis.

Although there exist divergent views on this topic, our group has routinely employed SDF (TUNEL assay) and other methods for identification of sperm DNA damage (Chromomycin A3, MitoTracker Green, Annexin V) during the investigation of male factor infertility over the last 10 years. We call this global assessment sperm DNA integrity (SDI), and during this time we analyzed some clinical implications, as below.

## (I) Are there age thresholds for increased SDI?

The effect of aging on SDF was evaluated in 2,178 semen samples. SDF deteriorated with aging (SDF:  $\leq 35$  years, 14.7%;  $\geq 36$  years, 16%;  $P < 0.05$ ), and this seems to be associated with mitochondrial damage (2). Sperm apoptosis, measured using Annexin V, is not correlated with aging (apoptosis:  $\leq 35$  years,  $19.1\% \pm 8.0\%$ ; 36–39 years,  $19.3\% \pm 7.9\%$ ;  $P > 0.05$ ) (2). Evidence suggests that there are declines in semen quality and male fertility associated with increasing male age. Advanced paternal age has been implicated in a higher frequency of miscarriages, autosomal dominant diseases, aneuploidy, and other disorders. Advanced male age has also been correlated with infant mortality (3–5). One plausible explanation for these outcomes is that older men may have more sperm with damaged DNA. Chromatin damage has been associated with male infertility, conception problems, and difficulties to sustain a pregnancy. There is also evidence linking sperm DNA damage

with the risk of mutations and birth defects (6–9). The age-related increase in sperm DNA damage suggests that delaying childbearing, not only for women but also for men, may jeopardize the couple's reproductive capacity.

## (II) Does varicocele affect SDI?

SDI is considered a marker of male fertility potential. High levels of SDF have been significantly linked to lower rates of natural conception and assisted reproductive pregnancies (1,10). Varicocele has been associated with increased levels of reactive oxygen species and decreased seminal antioxidant capacity, increased SDF and defective spermatogenesis in affected patients. A cross-sectional study was carried out with semen samples from 2,399 men, randomly selected from couples who attended an infertility clinic. A total of 16.3% (391/2,399) of the men had a varicocele (11). Physical examination was used to diagnose varicocele. Individuals with varicocele have increased SDF associated with an increase in abnormal mitochondrial activity and an increase in abnormal chromatin packaging (SDF with varicocele,  $16.3\% \pm 8.8\%$ ; SDF without varicocele,  $15.3\%$ ; OR: 1.02, 95% CI, 1.01–1.03,  $P = 0.03$ ) (11).

## (III) Is overweight/obesity associated with a decrease in sperm quality and impaired SDI?

Obesity appears to have a negative influence on the male fertility and reproductive function overall. Overweight and obesity have been associated with significant reductions in the levels of total testosterone, free testosterone, and sex hormone-

binding globulin, with elevated levels of estrogens (12-22). In addition, the build-up of suprapubic and inner fat may cause scrotal hyperthermia, which is a probable cause of elevated oxidative stress (13,18,23,24). Some studies have highlighted problems with sperm quality in patients with high body mass index (BMI), but the results are conflicting (12-26). SDI is another factor that may affect obese men; however, previous studies have shown controversial results regarding such an influence. BMI measures were carried out in a cohort of 1,824 men from couples who underwent infertility evaluations (27). High BMI negatively impacts sperm quality. In our study, high BMI was not associated with SDF, apoptosis, or protamination, but it was associated with increased mitochondrial damage.

Given the adverse consequences of obesity, the benefits of weight reduction should be discussed when counseling couples interested in fertility treatment. The exact mechanisms that mediate the effects of obesity on sperm mitochondrial damage require additional investigation. Possible medical actions would include counseling for weight reduction, antioxidants, and bariatric surgery.

Despite the clinical information acquired in recent years, important issues remain to be solved, as below:

- (I) The methods employed for determining SDF or SDI use several technologies to evaluate different DNA-damaging processes. It is not known exactly where the DNA is damaged and the degree of pathophysiological importance of the damaged site. The limits of normality are variable, making it difficult to analyze the cases individually;
- (II) Clinical interventions are not always conducted with the support of evidence-based medicine;
- (III) Only with the development of more precise and specific methods for DNA damage assessment we will be able to clarify the many unsolved issues concerning SDF and male infertility.

## Acknowledgements

None.

## Footnote

*Conflicts of Interest:* The author has no conflicts of interest to

declare.

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**Cite this article as:** Franco JG Jr. Sperm DNA fragmentation. *Transl Androl Urol* 2017;6(Suppl 4):S516-S518. doi: 10.21037/tau.2017.05.07