Editorial

Sperm DNA fragmentation: laboratory and clinical aspects

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The commentary by Drs. Khalili and Agha-Rahimi on the sperm DNA fragmentation (SDF) clinical guideline article by Agarwal *et al.* (1) recognized the presence of a negative association between high seminal SDF levels and fertility outcome specifically with assisted reproductive techniques (ART). Moreover, they have raised important concerns related to the lack of standardized SDF cutoff values and the influence of sperm preparation techniques during the course of ART on SDF levels. Furthermore, they brought into question, the exact treatment strategies that can be used for patients with high SDF. We certainly agree with these concerns which deserve further elaboration as seen below.

Many of the sperm preparation techniques that are performed during semen handling may indeed impose a risk of sperm DNA damage. This risk is believed to be mediated by the state of oxidative stress (OS) that occurs secondary to the exposure of sperm to exaggerated levels of reactive oxygen species (ROS) during sperm preparation. An understanding of the exogenous sources of ROS that take place during the course of ART is hence vital in order to prevent the development of SDF. Variables such as atmospheric oxygen concentration, illumination, centrifugation force, pH and temperature, culture media and cryopreservation are all implicated (2). Drs. Khalili and Agha-Rahimi have particularly tackled the influence of incubation time/temperature and culture media on SDF levels. It is believed that long incubation periods can influence sperm quality, measures of OS and SDF (3,4). It is therefore recommended that the time between sample production and the start of preparation is kept as short as possible and begin immediately after liquefaction. Incubation temperature is an important variable which could also influence sperm quality with increasing temperatures eliciting ROS-induced cellular damage (5). Recent studies have demonstrated an improvement in sperm quality (6) and a reduction in SDF measures (7) with samples stored at room temperature (23–25 ℃) in comparison to those stored at body temperature (37 ℃). Lastly, the sperm preparation medium used prior to ART may influence the quality of sperm and consequently the overall treatment outcome (8). The presence of metallic ions in culture media can potentiate increased ROS generation (9,10). Therefore, the use of antioxidants in culture media has been investigated and found to carry favorable effects on sperm quality (11).

While enquiring about treatment options available for patients with high SDF levels, Drs. Khalili and Agha-Rahimi have offered the implementation of lifestyle changes, use of sperm selection methods or testicular sperm for intracytoplasmic sperm injection (ICSI). Indeed, we have highlighted in the clinical guideline article (1), the importance of SDF testing in patients exposed to pollutants or who have modifiable lifestyle risk factors recommending it to reinforce these changes, predict fertility and as a monitoring tool for patient's response to intervention. Furthermore, patients with high SDF levels could also benefit from treatment with antioxidant supplements (12). Antioxidants have been thoroughly investigated in the treatment of male infertility. While a number of studies have confirmed a beneficial effect for antioxidants in reversing OS induced sperm dysfunction, fewer reports did investigate the effects of dietary antioxidant intake on

sperm DNA integrity (13-16). Specifically, vitamins C and E, beta-carotene, zinc, selenium and folic acid were found to be significantly helpful in this regard (14,15,17). One systemic review of 20 trials examining the effect of oral antioxidants on measures of sperm OS and SDF revealed that a significant reduction in these measures was reported in 19 out of the 20 studies selected. (18).

A variety of interventions have been proposed for patients with high SDF undergoing ART. Shortening of the abstinence time with frequent ejaculations (19,20), sperm selection using density centrifugation combined with swim-up technique (7,21), magnetic-activated cell sorting (MACS), physiological ICSI (PICSI) or intracytoplasmic morphologically selected sperm (IMSI) (22,23) and the use of testicular sperm have all been documented (24,25). Nevertheless, studies comparing the efficacy of these techniques in selecting sperm with better DNA integrity for ICSI are sparse. One retrospective analysis by Bradley *et al.* (26) investigated ICSI outcomes of 1,924 infertile men comparing results of patients with low SDF to those with high SDF who did or did not receive an intervention such as PICSI, IMSI or testicular sperm extraction/aspiration. In general, patients with high SDF who did not have an intervention had lower fertilization and poorer clinical outcomes compared with low SDF patients. In contrast, patients with high SDF who had an intervention had significantly improved clinical outcomes, similar to those with low SDF. Comparing the three interventions, the authors revealed that the highest live birth rate was detected in the testicular sperm subgroup (49.8%), followed by PICSI (38.3%) and IMSI (28.7%).

Finally, we agree that the presence of different SDF testing methods with lack of standardized cutoff values may still impose an obstacle to its routine use in clinical practice. Recent reports have raised this issue advising andrology laboratories to evaluate the type of SDF test they use and determine their own threshold values (27). Nevertheless, it is arguably rational to suggest that with the intended purpose of the clinical guideline article (1) to identify the proper indications of SDF testing, meaningful refinements of its practical methods and diagnostic thresholds could be achieved.

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Footnote

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

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