Commentary

Sperm DNA fragmentation testing in male infertility work-up: are we ready?

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We read with interest the guideline proposed by Agarwal and colleagues (1) on clinical utility of sperm DNA fragmentation (SDF) testing. The Authors stated that, despite the clear association between SDF and male fertility, the clinical implication of SDF is poorly understood. So, the aim of the guideline was to underline the actual indications of SDF testing and also to explain the management of patients with increased SDF. To achieve this purpose, the Authors examined original and review articles concerning the significance of SDF testing and arranged their manuscript into two main sections: in the first part they described the current tests for SDF evaluation, underlining their basic principles as well as the main advantages and disadvantages; in the second part they performed an evidence-based analysis of the utility of SDF tests under specific clinical scenarios, commonly found by urologists and reproductive specialists. In particular, in the clinical scenario #1 varicocele was analyzed: Agarwal and colleagues stated that SDF testing may allow to better select varicocelectomy candidates among patients with clinical varicocele and borderline to normal sperm characteristics. In clinical scenario #2 the authors considered the unexplained infertility, suggesting performing SDF testing in couples with recurrent spontaneous abortion (RSA) or before starting intrauterine insemination (IUI). Clinical scenario #3 described the relationship between SDF and assisted reproduction techniques (ART), recommending the SDF analysis in patients with recurrent ART failures. Finally, in clinical scenario #4 the influence of lifestyle risk factors on male fertility was considered, suggesting offering SDF testing to infertile men with evidence of this kind of exposure, in order to underline an eventual sperm DNA damage and/or to monitor the patient's response to treatment.

First of all, the paper underlines the relationship between SDF, male fertility and ART outcome: this association is also recognized by many authors (2-5). This relationship is particularly noticeable considering the clinical context in which the test is performed and the presence of variables of male/female origin: for example, in case of advanced maternal age, sperm chromatin integrity is an important feature, because in this condition the oocytes ability to repair the sperm DNA damage is limited (6,7). In 2015, also the Practice Committee of the American Society for Reproductive Medicine (ASRM) recognised the value of SDF testing, but underlined that data relating to the link between sperm DNA damage and ART outcomes are insufficient to routinely recommend the use of SDF testing (8). This paper refers to a Guideline created by ASRM in 2013 (9) to analyze specific clinical target areas in which SDF testing may be used to predict pregnancy rates: natural conception, IUI, IVF, ICSI and pregnancy loss. It was concluded that, for all these cases, the predictive ability of the SDF testing alone on ART outcomes is low and lacks validation. In our opinion, regarding the validation and inclusion of SDF testing in the male infertility work-up, some outstanding issues remain. First of all, the available SDF tests, well described in the text, provide different information about the sperm chromatin status (direct measures of the extent of DNA damage, or measure of DNA susceptibility to denaturation; detection of single or double stranded fragmented DNA; degree of protamination;

etc.). These factors could have different clinical implications: for example, the oocyte is able to repair DNA single strand breaks, but can make 'mistakes' in presence of DNA double strand breaks, producing genetic mutations that could modify or arrest embryo development (10). In addition, the recommended threshold values of SDF testing to discriminate between fragmented or not-fragmented semen samples display a high degree of variability. The reason is due to the different type of assays employed, to the standardization difficulties of the protocols, to the instrumentation used for the test (flow cytometry or microscopy) and to the condition of the analysed sperm samples (for example raw or treated). In particular, the choice of performing the SDF test on raw or treated semen is still a matter of debate. Several authors support the idea that the predictive ability of the sperm DNA damage test, performed on raw semen, decreases when spermatozoa are treated using routine semen preparation procedures, such as density gradient centrifugation (DGC) (11). The reason why raw sperm parameters have low prognostic value on ART outcomes, might be the "normalizing effect" of the sperm preparation technique, that may lead to the selection of a sperm population showing characteristics (morphology, motility, sperm DNA damage, etc.) very different compared to the native semen. Therefore, several authors argue that it would be very useful to evaluate sperm DNA damage in the right context: in raw semen in relation to natural conception and in post-treated samples with reference to ART (12). This issue was deepened in a recent meta-analysis of studies which tested the effect of SDF on ART outcome (13): the studies showed a significant association between DNA fragmentation and miscarriage rate, underlining that DNA damage in prepared semen had a stronger association than the raw semen. On the other hand, some authors argue that, in ART, native semen has higher or equal specificity and predictive ability than treated semen (14).

In our opinion, another very important question is: if the presence of a semen sample with a high proportion of DNA fragmentation is recognized, are we actually able to select the "healthy" spermatozoon among all? Currently, as described in this guideline, numerous strategies have been proposed to decrease the presence of sperm with fragmented DNA, reviewed in Tarozzi *et al.* (3), from the intake of oral antioxidants, to the selection of sperm with more targeted methodologies (e.g., magnetic-activated cell sorting and electrophoretic separation), to the selection techniques of spermatozoa directly during the microinjection process (PICSI, IMSI, birefringence), up to retrieval of male gametes directly from the testis. Several papers underline the effectiveness of these different strategies to select a sperm population with low level of fragmented DNA, but to date, none of these is a gold standard, being able to give us the opportunity to use a sperm devoid of DNA damage.

We believe that the most innovative contribution of this manuscript is the fact that provides clear indications of clinical situations in which the test can be used to benefit patients, by helping clinicians with clear information of practical use and easy to understand. In this regard, among the clinical practical applications of this test, we would like to suggest another possible use of the SDF testing, emerged for the first time in a recently published paper (15): the study showed that DGC, a routine sperm preparation procedure for IVF/ICSI, may produce an increase in sperm DNA damage in a number of patients, which negatively impact on pregnancy rate. So, Muratori and colleagues proposed SDF testing, before and after DGC, for all the patients who undergo assisted reproduction treatments and suggested the use of alternative sperm preparation procedures in case of increased DNA damage after DGC.

In conclusion, we agree with the authors that the use of SDF testing, along with conventional semen analysis, could provide a better knowledge of male fertility potential. On the other hand, it can be stated that SDF testing does not provide completely black or white results, therefore additional studies with standardized methods, correct study populations and larger number of cases are required to extend the clinical value of SDF testing and to routinely include this test in the male infertility work-up.

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Footnote

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

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