Commentary

Some relevant points on sperm DNA fragmentation tests

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The article by Agarwal *et al.* (1) published *in Translational Andrology and Urology* at the end of the past year, faces the important topic of the clinical utility of sperm DNA fragmentation (SDF) test.

Currently, a great debate is present in the literature regarding the possible routine use of SDF within male and couple infertility work up. The article by Agarwal *et al.* (1), besides reviewing the different methods that can be used to detect SDF, has the merit to provide clinical indications for some medical scenarios where the SDF tests may have relevance (summarized in the Table 2 of Agarwal's paper).

The most important problem emerging from the current debate in the literature regarding the clinical utility of SDF tests is the presence of several possible assays which are very different both in the procedure and in the type of damage they are detecting. Regarding these points, the article by Agarwal *et al.* (1) reports the main advantages and disadvantages of the different techniques used to detect SDF in Table 1 of Agarwal's paper. The issue of the type of damage revealed by the different techniques is very important and deserves a deeper investigation. Keeping in mind that the most important damage is the one impacting the reproductive outcomes, it is important to define better the relationship between the used test and the considered outcomes. This point has been faced in recent meta-analyses (2-5) where studies were grouped according to the methods. Interestingly, when the miscarriage rate is considered as endpoint both after assisted reproductive techniques (ARTs) and natural pregnancies, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method appears to be the most predictive, followed by sperm chromatin structure assay (SCSA) (2). Similar results were obtained in the meta-analysis by Osman *et al.* (4) where the considered endpoint was live birth rate after ARTs and where TUNEL was again the most predictive technique followed by single cell gel electrophoresis assay (COMET). According to this meta-analysis, when intracytoplasmic sperm injection (ICSI) was employed to perform ART, none of the tests was predictive of clinical pregnancy (4). In the meta-analysis performed by Cissen *et al.* (3), where clinical pregnancy after *in vitro* fertilization (IVF) and ICSI was considered as endpoint, only TUNEL and COMET showed a "fair" predictive value, whereas SCSA and sperm chromatin dispersion test (SCD) tests showed a "poor" prediction. Finally, the recent meta-analysis by Simon *et al.* (5) reports that TUNEL, COMET and SCD were predictive of clinical pregnancy after IVF and ICSI. Although the included studies in these meta-analyses are different, and female factor is neglected in most studies, all appear to agree on the fact that SCSA and SCD techniques are, respectively, not or poorly predictive of ARTs outcomes. The scenario appears different in studies on natural reproduction (6) and intrauterine insemination (IUI) (7), where SCSA technique results were found to be a good predictor of pregnancy. This result was confirmed in a recent study by Ribas-Maynou *et al.* (8) where also COMET, TUNEL and SCD were predictive of natural pregnancy. Overall, these studies confirm that the different techniques detect different types of damage. This conclusion is supported also by studies investigating the effect of sperm selection techniques on DNA integrity. It has been recently shown that the density gradient centrifugation (DGC) technique provokes an increase of DNA damage in highly motile selected sperm

(9,10). However, at variance with TUNEL, such increase does not appear to be detected by SCSA (9). Further studies will be necessary to understand whether also COMET and SCD are able to detect DNA damage after DGC. It must be considered that most studies evaluating SDF before and after DGC selection report the average pre- and post-DGC SDF values, possibly masking effects present in single samples (10). Post-selection DNA damage could highly affect outcomes of ART (10).

Another important consequence of lack of standardization and heterogeneity of the SDF detecting assays is that several threshold values have been proposed to discriminate pathological and normal conditions. This fact contributes to create confusion regarding the introduction of SDF tests in the diagnostic management of infertile men. Hopefully, a committee shall be soon organized to decide which is the gold standard method to evaluate SDF in the couple infertility work-up.

In the review by Agarwal *et al.* (1), the authors discussed the paper by Esteves *et al.* (11) which described a subpopulation of sperm with massive nuclear SDF, the so called "degraded sperm", which is overrepresented in varicocele patients. These authors suggested that patients with varicocele could be identified by the sole examination of semen specimens, provided the differentiation of sperm with fragmented and degraded DNA was performed. These data suggest that besides the type of damage detected, SDF tests should have the ability to focus on the clinically relevant sperm population. Our group has recently demonstrated that a cytometric sperm subpopulation [the so called "brighter sperm" (12)] is a better predictor of natural pregnancy (13). Similarly, focusing on the viable sperm fraction (14) is expected to retain higher clinical value as only viable spermatozoa participate in the fertilization process. Along the same line, it is expected that sperm selected for ARTs should be the clinically relevant sperm subpopulation for prediction of outcomes: evaluating SDF in the fraction used for oocyte fertilization should result indeed in higher prediction. However, whether SDF in selected sperm is more predictive of ARTs respect to neat semen is currently controversial (10,15,16).

Regarding the interesting point of clinical indications raised by the review of Agarwal *et al.* (1), it should be mentioned that, in addition to the clinical scenarios considered by the authors in Table 2 of the paper, emerging data in the literature suggest that other conditions may benefit from using SDF as diagnostic tool, including men with advanced age (17), diabetes (18,19), presence of inflammatory signs of the lower genital tract (20) and cancer (21-23). Concerning this latter condition, it has to be considered that both the presence of malignancy (as part of the paraneoplastic syndrome (24) and the chemo-radio therapies (23) required to treat cancer, affect DNA integrity of germ cells.

An important question that the clinicians face in case of a patient with high SDF levels is what to do next. Among the possible strategies, the clinician may choose to treat the patient to restore sperm DNA integrity, or to select the most appropriate ART treatment. Considering that testicular apoptosis and oxidative stress are the main mechanisms generating SDF (25,26), compounds able to target these two mechanisms are possible useful tools. Although some studies using antioxidants reported a positive effect in reducing SDF levels (27-29), no clear conclusions can be drawn about their effectiveness on reproduction outcome because these studies show several limitations (30). Similarly, although follicle stimulating hormone (FSH) has been used to target testicular apoptosis in several studies, whether the hormone is effective in reducing SDF levels remain to be defined because of the presence of non-responding subjects (31-35). The strategy based on selection of patients according to the FSH receptor (FSHR) genotype before treatment proposed by Simoni *et al.* (35) appears to be promising. In case of ART treatment, an issue often neglected by clinicians concerns the iatrogenic induction of DNA damage during *in vitro* sperm manipulation (9,10,36). Indeed, it is well known that SDF may proceed after ejaculation during *in vitro* incubations similar to those used to fertilize the oocyte by IVF (37-39).

In conclusion, on the road to introduce SDF in the male infertility work-up, more studies are needed addressing three important points: (I) establishing the gold standard technique for each reproductive outcome; (II) finding effective pharmacological treatments to decrease sperm DNA damage *in vivo*; (III) establishing correct strategies to prepare spermatozoa for ARTs to avoid iatrogenic damage.

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Footnote

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

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