

Implication of sperm processing during assisted reproduction on sperm DNA integrity

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The authors (Drs. Borini, Tarozzi and Nadalini), in their invited commentary (1) in response to the practice recommendations by Agarwal *et al.* (2), raised several valid arguments about sperm DNA fragmentation (SDF) testing. In our response to Borini *et al.*, we intend to share additional information on SDF for the readers to enrich this ongoing discussion.

A number of guidelines from various professional societies are currently available concerning evaluation of infertile male patients. The Practice Committee of the American Society of Reproductive Medicine (ASRM) published a clinical practice guideline on the value of SDF testing in 2015 (3). The Committee Opinion does not recommend the routine use of SDF based on the limited data which underlines the relationship between abnormal DNA integrity and reproductive outcomes. However, the more important message from the Committee Opinion is that the effect of abnormal SDF on the results of intrauterine insemination (IUI)/in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) is evident in view of the expanding literature on the topic. Actually, we agree with the ASRM guideline that states the low predictive ability of SDF testing alone on assisted reproductive technology (ART) outcomes in the context of a complex reproductive system in human. It is unlikely that the result of dynamic interaction among multiple confounding factors, both in natural conception and ART, can be confirmed by a single test. This is the main reason for

practice recommendations (2), as proposed by Agarwal *et al.*, in establishing the role of SDF tests clinically based on the best available evidence. We believe that the observation from a wider application of the test clinically, particularly in the scenarios suggested in the practice recommendations but not limited to it, will improve our understanding in selected utilization of SDF tests in suitable patients in a cost-effective manner.

Borini *et al.* highlighted important limitations of SDF tests, such as the lack of standardization and different nature of SDF detected by various methods. But it is also prudent to recognize the efforts from various researchers worldwide in response to the pitfalls of SDF tests over recent years. There are numerous studies in the literature comparing various SDF tests (4). The results of these assays are generally not comparable due to the different aspects of SDF measured, as mentioned by the authors. Nonetheless, the tests are interrelated to a greater or lesser extent by reflecting the overall quality of the specimen and the properties of the sperm DNA, and may point to a common origin of damage (5). Moreover, the predictive value of SDF in both natural pregnancy and ART outcomes has been consistently reported from various centers by utilizing different testing methods in a wide range of patients (6). In fact, various SDF tests including sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays have correlation coefficients

ranging from 0.3 to 0.7 signifying a moderate correlation (7,8). Despite the current lack of a single gold standard SDF test, the paramount importance of standardization of a particular SDF test method is illustrated by the very high degree of accuracy between different laboratories when using an optimized protocol and test kits for TUNEL assay (9,10). Therefore, SDF tests are potentially useful clinically provided an optimized protocol and good quality control are available in a specialized andrology laboratory, irrespective of the different testing methods used.

The notion of “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” (1) is unfortunately not supported by current evidence. DNA fragmentation index (DFI) of density gradient centrifugation (DGC)-processed sperm measured by SCSA did not predict ART outcome in contrast to neat samples, despite the observation of a reduction in DFI (11). No association between sperm SCSA DFI after swim-up and fertilization, implantation and pregnancy rates could be demonstrated in another study (12). In addition, DGC has been reported to result in increased SDF especially when higher centrifugation force, longer duration and Percoll gradients were used (13,14). The use of processed semen samples for SDF testing remains controversial and further studies are warranted.

Finally, the authors reported their experience in using DGC as the sperm preparation procedure for IVF/ICSI. The result showed that approximately 50% of the patients in their study demonstrated increased SDF after DGC leading to lower pregnancy rate (15). The study result concurs with previous finding that sperm from infertile patients with higher baseline SDF are prone to further damage after DGC (14). Zini *et al.* have reported an improvement in sperm motility after DGC in both fertile and infertile patients, in contrast to SDF which showed a significant increase from 15% to 25% in spermatozoa recovered after DGC in infertile patients (14). The susceptibility to DGC-induced stress in sperm from infertile men may be explained by the higher content of reactive oxygen species (ROS) in the semen samples (16). While lipid peroxidation of sperm membrane and resultant impaired motility is less likely after DGC (17), the higher proportion of abnormal spermatozoa in samples of infertile men may act as a significant source of ROS. ROS production is augmented by serial centrifugation resulting in further loss of sperm DNA integrity during DGC (16). Although the hypothesis needs verification by further clinical studies, it provides us

with an insight into the potential drawback of the current sperm preparation techniques during IVF/ICSI cycles. The recovered spermatozoa after DGC from infertile men with intact sperm motility are still able to fertilize the oocyte despite the presence of increased SDF (17). However, the deleterious effect of high SDF on embryo development and miscarriage is of concern. The unknown long term consequence of a successful pregnancy with very high levels of DNA damage is even more worrisome (6). Therefore, caution should be taken when utilizing DGC particularly in patients with high SDF in neat sample. Further studies on the safety of other sperm preparation techniques, including swim-up, sequential DGC and washing, glass wool filtration, and magnetic cell separation, is also needed. The possible etiology related to ROS has made the use of antioxidants in the wash medium an attractive option. The use of sperm selection techniques, for example hyaluronic acid binding, sperm magnetic sorting and high magnification microscopy on DGC-processed sample may select “healthier” spermatozoa for ICSI. However, all current sperm selection techniques are unable to completely remove sperm with DNA damage (18). In this context, we argue against the use of post-DGC SDF as a cost-effective clinically useful prognostic test to assess IVF/ICSI outcomes. The result of SDF test on neat samples before processing has been shown to correlate with ART outcomes (11) and a high SDF before DGC predict further impaired SDF after processing. Therefore, addition of post-DGC SDF tests may not provide more information than a single SDF test on neat sample. Further studies about the implication of sperm processing on SDF are eagerly awaited.

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Footnote

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

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