Editorial

The complex nature of the sperm DNA damage process

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Dr. Menezo and colleagues (1), in their commentary regarding the practice recommendations for sperm DNA fragmentation (SDF) testing based on clinical scenarios by Agarwal *et al.* (2), pointed to the importance of distinguishing between SDF and sperm nucleus decondensation (SND) as these are independent processes. The authors reasoned that while the oocyte has a limited capacity to repair DNA strand breaks, it is poorly equipped to repair nucleus decondensation. They go further by listing conditions more likely to be associated with one (e.g., lifestyle factors and SDF) or the other (e.g., IVF/ICSI failure due to early embryo arrest and nucleus decondensation) and the tests best suited to measure SND.

In our reply, we provide a brief overview of the last stage of spermatogenesis to help readers comprehend the problem of SND and DNA fragmentation. Moreover, we discuss in further detail the differences among the existing tests for sperm DNA damage assessment.

Upon meiosis completion, the haploid round spermatids initiate the final differentiation stage termed spermiogenesis. It involves no cell division but rather a complex series of cytological changes leading to the formation of spermatozoa (3). In humans, maturation of round spermatids to spermatozoa is characterized by four main events, i.e., (I) position of the nucleus in an eccentric location and condensation of the nuclear DNA (chromatin compaction) that results in nuclear size reduction; (II) formation of the acrosome from the Golgi complex; (III) tail formation from a pair of centrioles lying adjacent to the Golgi complex and the aggregation of mitochondria; (IV) cytoplasm phagocytosis by Sertoli cells (4). The final result is the production of highly specialized cells with fully compacted chromatin.

As mentioned above, histone–protamine transition under normal conditions results in a greater compaction of the sperm DNA molecule than that observed in somatic cells. During this transition, the DNA molecule would have been subjected to a forced twisting of the DNA molecule hadn't controlled DNA nicking taken place (5). This structural DNA nicking necessary for histone-to-protamine transition seems to be facilitated by topoisomerase II. Hence, any mechanism affecting the process of protamination can result in SND. When sperm maturation is completed, checkpoint-like mechanisms may trigger abortive apoptosis of such defective sperm, but the phenomenon is not universal, and sperm with abnormal chromatin compaction can be released in the ejaculate (5). Therefore, SND refers to defects in chromatin compaction (e.g., protamine mispackage via defective DNA-protein crosslinking), which is intrinsically associated with the later stage of spermatogenesis.

On the contrary, SDF specifically refers to the breaks occurring at the DNA strands, and they are termed single-strand (ss) or double-strand (ds) breaks (5,6). SDF often results from oxidative stress in the male reproductive tract (5,6). Excessive reactive oxygen species (ROS) can be generated in the epithelial cells of epididymis under physicochemical stressors such as high temperature and environmental conditions. Activated leukocytes and defective sperm can also release large quantities of ROS (5). As a consequence, redox processes using various pathways, including hydroxyl radical, nitric oxide, and activation of sperm caspases and endonucleases, affect not only the sperm

membrane but also nuclear and mitochondrial DNA, thus explaining the higher positivity for SDF in live ejaculated sperm of infertile men than fertile counterparts (5). In general, oxidative stress produces single-strand DNA breaks, but apoptotic events leading to double-strand breaks or cell death may take place as a result of SDF generated by ROS (5,6).

Sperm DNA damage is a broad term that encompasses both DNA fragmentation and nucleus decondensation (5). Although we agree with Menezo and colleagues that SND and SDF are independent processes, the theories to explain the origin of SND and SDF are not mutually exclusive, and a synergistic effect among the different effectors is likely to contribute to the accumulation of DNA damage. Importantly, defective chromatin compaction makes the DNA more vulnerable to damage by ROS, and as a consequence SDF may ensue (5).

In general, the methods for assessing sperm DNA damage can be grouped into three categories, namely, (I) assays that measure DNA fragmentation by incorporating DNA probes or modified nucleotides at the site of breaks; (II) assays that measure both the existing breaks and those generated after DNA denaturation; and (III) assays that indirectly measure the level of chromatin compaction, i.e., nucleus decondensation (6). While TUNEL belongs to the first group, sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) test and Comet are examples of the second group (6). Lastly, aniline blue and toluidine blue are methods that indirectly assess chromatin compaction (decondensation) (6). However, contrary to the authors' suggestion that SCD measures both fragmentation and decondensation, the SCD assay is not a method for assessing SND. The test is based on producing a controlled and species-specific DNA denaturation to produce single-stranded DNA stretches from any DNA break, coupled with controlled species-specific protein depletion (7,8). The resulting images, i.e., sperm with and without halos of dispersed chromatin around a dense core, represent those with normal and fragmented DNA, respectively.

The predictive value of these methods concerning infertility and assisted reproductive technology (ART) outcomes, assuming proper execution and quality control, depends on the quantitative (degree) and qualitative (location) nature of damage and the oocyte DNA repair capacity (5). In ART, other factors likely to modulate the reproductive success include patient characteristics, iatrogenic sperm damage after semen collection, techniques for sperm selection, and fertilization strategy (IVF or ICSI) (5,6,9). Contrary to Menezo *et al.* suggestion that SND should be incorporated into the evaluation of male infertility—this in addition to SDF—we however, believe that at present SDF should be the biomarker of choice for assessing sperm DNA damage. The reasons stem from the ubiquity of oxidative stress among males facing infertility and the overwhelming evidence of the detrimental effect of oxidative-induced SDF to both fertility and ART outcomes (10-17).

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Footnote

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