Editorial

More good than harm should be expected when Testi-ICSI is applied to oligozoospermic men with post-testicular sperm DNA fragmentation

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We read with interest the commentary by Dr. Paul Turek (1) contextualizing the use of sperm DNA fragmentation (SDF) testing for male infertility in response to the recently published practice recommendations for SDF testing based on clinical scenarios by Agarwal *et al.* (2).

We certainly concur with the author regarding the limitations of conventional semen analysis parameters as surrogate measures of male fertility potential (3,4) and that SDF testing is one of the most relevant advancements to the andrological evaluation of male infertility (5,6).

Moreover, Dr. Turek critically analyzed the use of testicular in preference over ejaculated sperm for intracytoplasmic sperm injection (ICSI), which has been presented by Agarwal *et al.* as an alternative to overcome infertility in men with elevated levels of SDF undergoing ICSI (2). In his commentary, the author caution against the indiscriminate use of testicular sperm for ICSI (Testi-ICSI) and rationalize his arguments based on the following premises: (I) there is no indication of Testi-ICSI in cases of failed IVF/ICSI cycles with ejaculated sperm normal or untested SDF; (II) the use of Testi-ICSI in cases of severe oligozoospermia without evidence of sperm DNA damage lacks supportive evidence; and (III) testicular sperm has higher chromosomal aneuploidy rates than ejaculated sperm.

Along these lines, we wish to add some comments that may help readers better understand the matter concerned. Foremost among all is perhaps the fact that the available evidence favoring the use of Testi-ICSI seems to be limited to men with elevated SDF rates in the neat ejaculate. In this scenario, others and we have shown that the rates of SDF are markedly lower in testicular sperm than ejaculated sperm (7-9). We have studied oligozoospermic (5–15 million spermatozoa/mL) men presenting with persistent high SDF (>30%) despite continuous use of oral antioxidant therapy for 3 months and found that SDF rates were fivefold lower in the testis (8.3%±5.3%) than in the semen (40.7%±9.9%; P<0.001) (7). In our study, SDF was assessed using the sperm chromatin dispersion (SCD) method combining a dual fluorescent probe to target both the DNA and proteins that allow discrimination between spermatozoa and other cell elements in testicular suspensions (10).

The biological plausibility of reduced SDF in the testis relies on three essential aspects. First, chromatin compaction is still ongoing during epididymal transit. Second, excessive reactive oxygen species (ROS) can be generated in the epithelial cells of epididymis under physicochemical stressors such as high temperature and environmental conditions (11-13). Lastly, certain endonucleases can cleave DNA of mature live sperm (14). As a result, sperm DNA damage may ensue through different pathways, including hydroxyl radical, nitric oxide, and activation of sperm caspases and endonucleases, thus explaining the positivity for SDF in live ejaculated sperm of infertile men (15). This oxidative-induced damage to the sperm chromatin can be potentially avoided in ICSI candidates provided the epididymis is

bypassed.

Notwithstanding, the use of testicular sperm not always overcomes the problem of SDF. Notably, SDF may also occur in the seminiferous tubules as a result of apoptosis or due to defects in chromatin remodeling during spermiogenesis (16). Intratesticular apoptosis induced by impairment in sperm maturation lead to early DNA damage; these spermatozoa traverse the genital tract without being further damaged by oxidative stress (16). Consequently, the advantage of testicular sperm over ejaculated sperm as regards decreasing SDF is likely to be restricted to post-testicular SDF. As suggested by Dr. Turek, and discussed below, it is important to evaluate the male partner of an infertile couple before embarking on assisted reproductive technology (ART). In this context, a comprehensive male infertility evaluation including SDF testing allows not only diagnosing and eventually treating the underlying condition associated with SDF but also identifying the best candidates for Testi-ICSI.

For instance, infertile men with varicocele usually have higher SDF than counterparts without varicocele (12). In these men, reactive oxygen and nitrogen species are released not only in endothelial cells of the dilated pampiniform plexus and testicular cells (developing germ cells, Leydig cells, macrophages and peritubular cells) but also in the principal cells of the epididymis (17). The epididymis can be the origin of SDF in other conditions as well, including infectious and inflammatory states that may contribute to chronic epididymal dysfunctions and spermatogenesis defects associated with residual cytoplasm and defective protamination. The former can be observed in spinal cord injury (18), post-vasectomy reversal (19), and clinical or subclinical epididymitis (20). In these cases, SDF may result from excessive ROS production by spermatozoa themselves in response to a more prolonged epididymal transit or infiltrating polymorphonuclear leukocytes, or both. The latter can be genetically determined or idiopathic, and SDF results from the higher susceptibility of DNA to post-testicular degradation by endonucleases (21). Also, oxidatively-induced SDF can also occur post-ejaculation for a strong association exists between the presence of male accessory gland infections and seminal ROS levels, and between smoking and excessive seminal plasma leukocytes and ROS; both conditions have been associated with high SDF (22,23).

In the study mentioned above involving 147 oligozoospermic patients with elevated SDF, we have shown that the number needed to treat (NNT) by testicular compared to ejaculated sperm to obtain an additional live birth per fresh transfer cycles was 4.9 (95% CI, 2.8–16.8) (7). In other words, we could potentially avoid one out of five oocyte retrievals in such couples. Although this simplistic estimation does not consider the additional contribution of frozen embryos in terms of cumulative live birth rates, the fertilization of an oocyte by a genomically intact testicular spermatozoon may improve the chances of creating a normal embryonic genome that will ultimately decrease the likelihood of miscarriage, which has been more often reported in ICSI cycles with high levels of SDF (24).

Despite the higher aneuploidy rates in testicular sperm compared with ejaculated sperm, as indicated by Dr. Turek, this proportion is still relatively small [approximately 12% in testicular sperm versus 6% in ejaculated counterparts (25)] and are yet to be confirmed in large series of men with oligozoospermia. Notwithstanding, it might be argued that ICSI candidates represent a particular category of patients that would be unlikely to attain natural reproduction. Therefore, a small increase in the risk of having health issues in the offspring could be acceptable in return of a confirmed beneficial effect of Testi-ICSI, provided the actual number of affected individuals were extremely low.

Lastly, although sperm retrievals are invasive interventions, the reported complication rates are very low and often minor (26). The most problematic adverse effect is reduction in testosterone production, which has been reported after large biopsies or repeated procedures in some men with nonobstructive azoospermia (27). On the contrary, from a holistic standpoint, we argue that less invasive treatments for the men (i.e., ICSI with ejaculated sperm) might represent more invasive treatments for the female (i.e., repeat oocyte retrievals) if fewer pregnancies and more miscarriages are obtained with ejaculated sperm in cases of high SDF.

To sum up, we believe there is a rationale for the use of testicular sperm for ICSI in men with high SDF due to the improvement in live birth rates. But at present, the method should be reserved for oligozoospermic men with post-testicular sperm DNA damage who have failed less invasive treatments for known and unknown causes of sperm DNA damage.

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Footnote

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

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