Commentary

A few more fragments: putting sperm DNA integrity testing into clinical practice

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While routine semen analysis has been the cornerstone of the male fertility evaluation, we know that it is far from a perfect predictor of fertility, and there will be some couples who struggle to obtain a live birth despite the male having a normal semen analysis. Indeed, the World Health Organization semen analysis normal reference ranges were based on samples from fertile men (1). The LIFE study, a prospective observational cohort study of 501 couples, which looked at the relationship between routine semen parameters and time to pregnancy, found that on multivariate analysis, none of the routine semen parameters (volume, concentration, motility or morphology) were associated with time to pregnancy. Only male age and female body mass index (BMI) were associated with the time to pregnancy (2). From both this data and others, we know that the standard semen analysis is not a perfect predictor for male infertility (3). These limitations have led to the investigation of sperm DNA fragmentation as an adjunctive test to guide the management of the sub-fertile couple.

There are a variety of testing methodologies for DNA fragmentation, and the first table in Dr. Agarwal's paper beautifully summarizes these. However, it should be noted that for each of the four clinical scenarios presented, there is some heterogeneity in the DNA fragmentation assay used. In addition, for each of these assays, there is a different threshold for "abnormal", and according to the 2015 ASRM guidelines, the threshold for an abnormal DNA fragmentation by SCSA is ≥25–27% and ≥30% for TUNEL assay (4). This does not account for female factors or other co-existing male factors, which may also account for some of the controversy that surrounds DNA fragmentation testing. To this end the ASRM states that "the routine use of DNA integrity tests in the clinical evaluation of male-factor infertility is controversial" (4). However, the scenarios presented in this article represent scenarios in which DNA fragmentation testing should be considered. In addition, even with the above-mentioned limitations, the patterns noted in this article do emerge as consistent themes. The question then becomes, how to best treat men who are found to have elevated DNA fragmentation levels.

The most basic intervention is antioxidant therapy. A multitude of antioxidants have been investigated, with varying doses and combinations. One study of 20 men with varicoceles treated with three months of antioxidants found a 22.1% reduction in sperm DNA fragmentation after treatment (5). An additional study of 20 men with asthenoteratozoospermia treated with three months of oral antioxidants, whose ejaculated sperm was then incubated for several time periods in antioxidants, showed a significant improvement in DNA integrity at all time points (6). A 2014 Cochrane review of antioxidants for male subfertility found that, based on low quality evidence (from randomized studies), antioxidant therapy may improve clinical pregnancy and live birth rates. Only two studies were included that looked specifically at DNA fragmentation, however, both of these had a positive response to antioxidant therapy (7). One of these was a study of 64 infertile men with high DNA fragmentation, who had an improvement after treatment with Vitamins C and E, although there was no change in the semen parameters (8). A subsequent study found that

treatment with docosahexaenoic acid resulted in a lower DNA fragmentation rate when compared with placebo (7).

As the authors have discussed, "interventions" that may be performed for men with elevated DNA fragmentation rates include optimization of lifestyle factors and environmental exposures, varicocele repair (when present) and testicular sperm extraction (TESE). In men with bacteriospermia and/or leukocytospermia, treatment with antibiotics also seem to improve DNA fragmentation rates (9,10).

As the authors have included a detailed review of, a growing body of evidence shows that DNA damage occurs during the epididymal transit of sperm (11). Accordingly, the use of testicular sperm for intracytoplasmic sperm injection (ICSI) has been shown to translate to improved live birth rates. Some groups suggest that sperm DNA fragmentation testing be a routine part of the male factor work-up, and when it is elevated that ICSI is indicated (12). Since the time of this publication, an additional manuscript has looked at reproductive outcomes and men with elevated sperm DNA fragmentation. Elevated sperm DNA fragmentation was correlated with a variety of negative reproductive outcomes, and the use of testicular sperm for ICSI improved these. Pregnancy rates were: 24.9% for men with elevated DNA fragmentation rates without any type of intervention (TESE), 40.6% for men with low DNA fragmentation rate, and 49.8% for men with elevated DNA fragmentation and TESE/ICSI (13). This was the second study to correlate live birth rates with the use of testicular sperm in men with elevated DNA fragmentation rates. The first study found a live birth rate of 46.7% with TESE/ICSI and 26.4% in ICSI using ejaculated sperm, with a DNA fragmentation rate of 8.3% in testicular sperm versus 40.7% in ejaculated sperm (14). The evidence continues to accumulate for the utility, and indeed indication, for TESE in men with elevated DNA fragmentation rates to provide healthier sperm to be used in ICSI. This has been shown to be true for normozoospermic men (13), oligozoospermic men (14) and severely oligozoospermic men (15).

In addition, sperm cryopreservation has been shown to have deleterious effects on sperm DNA by inducing DNA fragmentation and oxidation. In a study of 15 cryopreserved semen samples (5 with normal and 10 with abnormal semen analyses), an elevation in DNA fragmentation rate was seen after freezing (33% *vs.* 21%), even with the use of a commercial cyroprotectant (16).

Therefore, a fresh TESE/TESA is indicated for men with elevated DNA fragmentation rates at the time of ICSI. The use of sperm obtained by TESE has been shown to improve fertilization rates, blastocyst transfer outcomes, fetal heart pregnancy rates and now live birth rates (13,14). While most of these data are based on relatively small series, they are consistent. This is a concept that should be considered the standard, unless future date proves otherwise. ICSI is both expensive and emotionally draining for patients, and we want to set the stage for successful cycles in these couples.

So how should we manage the male with elevated DNA fragmentation? Inquire about lifestyle and occupational factors. All of these men should be put on an antioxidant regimen. The correction of any varicoceles present should be considered. Treat any leukocytospermia or bacteria present within the ejaculate. All of these are done with the goal of providing healthier sperm for a natural pregnancy. If the DNA fragmentation continues to be elevated, plan for a ICSI cycle with a fresh TESE/TESA. In this way, we truly are optimizing the male factor to provide these couples with the best chance for what they want most, a live birth.

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Footnote

Conflicts of Interest: The author has no conflict of interest to declare.

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