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# INVITED COMMENTARY

Male Fertility

## Use of testicular sperm to combat the negative effects of DNA fragmentation

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Numerous approaches to the detection of novel biomarkers for male fertility have been proposed. As reviewed in the manuscript by Bieniek *et al.*[1] analysis of seminal plasma has yielded numerous possible candidate biomarkers. Since semen analysis is a critical component in the initial workup for infertile males, seminal fluid biomarkers inherently have several distinct advantages. For most individuals, the sample is relatively easy to produce and, given an appropriate volume, can be separated into multiple aliquots for separate tests. Disadvantages include the fact that seminal fluid is a composition of excretions from multiple glands, including the seminal vesicles and prostate.

DNA fragmentation has been investigated for a decade with regards to not only its potential as a biomarker, but also as a measurable value by which to postulate fertility outcomes and record spermatogenic damage from reactive oxygen species (ROS).[2,3] Simply put, DNA fragmentation is a measure of DNA integrity assessed through different methods such as the Comet and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) tests.[4]

ROS-induced DNA damage, manifested as increased DNA fragmentation, occurs primarily during posttesticular transport of spermatozoa through the epididymis. As such, a strong relationship between ROS and DNA fragmentation has been established.[5,6] Indeed, DNA damage can be evoked *in vitro* by exposing mature sperm to high levels of ROS.[7,8] Highly abnormal (>30%) DNA fragmentation rates have also been identified in ~8% of infertile men with a normal semen analysis;[9] these data highlight the potential importance of this seminal biomarker.

A role for testicular sperm retrieval as treatment for DNA fragmentation was hypothesized in a study that followed four couples with multiple *in vitro* fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) cycle failures using ejaculated sperm. Pregnancy was subsequently achieved using testicular sperm aspiration.[2] While the authors did not directly examine DNA fragmentation, the theorized relationship was provocative.

When considering natural pregnancies, high seminal DNA fragmentation rates treated with multivitamins and anti-oxidants have failed to show significant improvements in fertility. Conversely, work by Greco *et al.* examined and compared the characteristics and outcomes of ejaculated and testicular spermatozoa from 18 men undergoing IVF/ICSI.[3] The authors found that DNA fragmentation (via TUNEL) was lower in testicular sperm compared to that of ejaculated sperm.[2] Moreover, IVF/ICSI with testicular sperm yielded improved outcomes in men with high rates of DNA fragmentation.[3] Following these results, it was recommended that in men with previous IVF/ICSI failures using ejaculated sperm containing high levels of DNA fragmentation, testicular samples should be considered to improve outcomes.[3,10]

Another point to remember is that varicoceles, known contributors to male factor subfertility, can increase ROS and DNA fragmentation rates. Microsurgical repair of the varicocele can obviate these negative semen analysis/seminal biomarker characteristics.[11] It is thus tempting to speculate that in men with prior IVF/ICSI failures and concurrent varicoceles, repair followed by a testicular sperm harvest at least 3 months following the surgical procedure could yield the most successful outcomes. Trials are encouraged and would no doubt lead to interesting results.

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