New diagnostic tests for male infertility

Two technologies will help clinicians direct therapies for complicated infertility patients

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Despite the ongoing controversies regarding the accuracy and predictive power of routine semen analysis, it continues to be used by many clinicians worldwide as the de facto test for male infertility (Int Braz J Urol 2014; 40:443-53). In its best practice statement for the evaluation of the infertile male, the AUA has proposed the use of advanced tests of sperm function in certain patients to enhance the diagnostic accuracy of semen analysis, specifically in cases of unexplained infertility, recurrent pregnancy loss, or failure of intrauterine insemination (IUI) and in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (The Optimal Evaluation of the Infertile Male. AUA Best Practice Statement, 2010).

As explained in this article, these new tests have the potential to improve our ability to better diagnose and treat complicated male infertility patients.

Oxidative stress

Oxidative stress is thought to contribute to 40%-80% of male infertility (Fertil Steril 2003; 79:829-43) and arises as a consequence of excessive production of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms (figure 1) (Curr Med Chem 2001; 8:851-62). Although small amounts of ROS are important for normal sperm function, an excess of these highly reactive molecules can cause damage to the lipid-rich plasma membranes and the integrity of DNA within the sperm nucleus, as well as impaired motility and spermatozoa apoptosis (Fertil Steril 2003; 79:829-43).

Antioxidants such as some vitamins and minerals combat these overproduced ROS. In addition to excess ROS, oxidative stress can be due to insufficient concentrations of antioxidants as well. Over the last decade, research has provided growing support for the fact that oxidative stress leads to abnormal semen parameters. In addition, more and more laboratory tests are now available to measure this oxidative stress. Therefore, it would be reasonable to potentially screen all infertile men for the presence of increased ROS levels. Specialized training and equipment, the lack of cost-effective assays, and, perhaps most importantly, the lack of a universally accepted analytical methods have prevented ROS testing from being included as part of the routine infertility work-up.

ROS can be measured both directly and indirectly. Chemiluminescence is probably the most common way to directly measure ROS in sperm currently and can quantify both intracellular and extracellular ROS. It uses a luminometer in conjunction with a chemiluminescent probe such as luminol, which can also be used to measure a total antioxidant capacity (TAC). The results can be expressed as a ROS-TAC score, which can give an indication of the combined oxidant and antioxidant activities of seminal constituents. Unfortunately, this test requires special equipment, training, and is costly for widespread clinical use.

Due to these limitations, nitroblue tetrazolium (NBT) has been put forth as a cost-effective alternative. NBT interacts with free radicals and is converted to a blue pigment that can then be measured with light microscopy. This test suffers from a lack of standardization and low inter- and intra-observer reliability.

The new MIOXSYS Analyzer (figure 2) used at our institution measures the so-called oxidation-reduction potential (ORP). ORP measures...
the balance of all oxidants and antioxidants in the specimen and gives a complete picture of the oxidative stress. This test can be performed in less than 5 minutes. It requires no specialized training and may possibly replace the more complex and traditional oxidative stress tests without sacrificing the reliability. Elevated ORP levels correlate well with infertility, with a significantly higher ORP seen in infertile patients than in fertile controls.

In a recent study, ORP was able to detect at least one abnormal sperm parameter with a sensitivity of 70.4% and a specificity of 88.1%. It had an 88% sensitivity and 91.2% specificity when detecting oligozoospermia (Urology 2017; 104:84-89). Given the increased recognition that oxidative stress plays an important role in male infertility, development of reproducible and cost-effective techniques in measuring oxidative stress may help in tailoring our treatments for infertile couples.

DNA fragmentation

Researchers have turned their attention to the genetic contents of sperm, as embryo development depends in part on the inherent integrity of sperm DNA. DNA integrity testing is relatively new to the armamentarium for fertility specialists. While originally described in 1993, it failed to gain traction as a clinical test due to lack of availability and standardization. Sperm DNA is highly compacted by binding tightly to protamine. A certain degree of sperm DNA damage can often be repaired by the oocyte’s antioxidant enzymes. When damage exceeds the repair capability of the oocyte, deleterious effects of sperm DNA fragmentation (SDF) may result, such as miscarriage and poor embryo development (Hum Reprod 1999; 14:1039-49).

Because of this, more attention has turned to testing for SDF. This test does not evaluate the actual genetic code of the DNA within the sperm, but rather the overall superstructure of the DNA strands. There are many different ways to test for SDF, each with pros and cons. We will focus our discussion on the most commonly used tests as well as what is considered the gold standard, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Asian J Androl 2016; 18:205-12).

The acridine orange (AO) test uses a fluorescent dye that emits a different wavelength of light based on whether it is bound to double-strand DNA (normal) or single-strand DNA (abnormal). This test is fast, simple, and inexpensive but limited by inter-laboratory variations and lack of reproducibility. Another relatively simple test, the “halo” test, has similar pros and cons as the AO test, but evaluates the characteristic halo of dispersed DNA loops around sperm with non-fragmented DNA.

The latest technology using TUNEL detects Please see INFERTILITY TESTS, page 12
Q: What percentage of prostate cancer cases are caused by genetics?
A: Most cases of prostate cancer are caused by genetic alterations. The problem is that when you break it down to very specific, identifiable, inherited prostate cancer risk genes, we have very few at the present time. All tumors are driven by genetics, but when you look at specific inherited risk, our current level of understanding is that about 10% to 15% of patients can have a clearly identifiable inherited component to their prostate cancer.

Q: This is a very active area of research. Please talk about what’s new and exciting in the world of prostate cancer genetics.

A: The completion of the Human Genome Project in 2003 opened the door for not only basic science advances but drove the clinical applications of genomic and genetics. Urologists have recently become very familiar with the genomics of prostate tumors studying somatic mutations to help guide treatment decisions. The area we are now becoming interested in is known as germline testing or the study of inherited genetics. We’ve been able to identify more and more inherited genetic alterations in medicine. The traditional ones that we have the most familiarity with are the BRCA1 and BRCA2 abnormalities associated with hereditary breast and ovarian cancer. But it turns out that a significant number of men can also have BRCA1 or BRCA2 genetic alterations that can confer an increased risk of prostate cancer. Several newer genes such as HOXB13 and ATM have also been identified as being associated with prostate cancer. Importantly, we’re recognizing that not only can prostate cancer run in families but it also can be related to breast cancer, ovarian cancer, pancreatic cancer, melanoma, and Lynch syndrome in other family members. This area of research is giving us some direction on how urologists can think about approaching our patients concerning the need for more detailed family histories.

Lastly, genetic panels are now being offered by commercial laboratories specifically for prostate cancer. Urologists need to be aware that these panels are out there, and the best way to utilize these genetic testing panels is something we’re all going to have to learn.

Q: You recently served as co-chair for the Prostate Cancer International Consensus Conference on the role of genetic testing for inherited prostate cancer risk. Could you talk about the rationale for this meeting and what was discussed?
A: With all the recent advances in genomics and genetic testing, we realized that there was a significant need for more detailed family histories.

Conclusion
Both oxidative stress testing and DNA fragmentation testing are relatively new technologies that will help clinicians choose the most appropriate therapies for their patients. While no large-scale randomized studies have been performed yet, emerging evidence on both are promising. We believe they will eventually become commonly used in clinical practice for the management of male infertility.