DNA methylation profiling of peripheral blood samples is a promising new approach to screen for male infertility

Male infertility is a complex, multifactorial, and heterogeneous condition. In ~15% of couples, infertility results from an identifiable genetic defect. However, in 40% of couples, male infertility is likely to be caused by an unknown genetic abnormality. Consequently, many men evaluated for infertility may not ultimately be diagnosed beyond the phenotype of their infertility, such as azoospermia or maturation arrest. Emerging research into the mechanisms underlying idiopathic male infertility promises new opportunities to provide these men with more specific diagnoses. Although semen analysis remains an integral tool in the evaluation of the male partner, most genetic defects associated with male infertility are not incorporated into current clinical testing (1). In addition to incorporating these known defects into clinical tests, an understanding of defects outside of DNA-level changes, such as transcriptional and epigenetic aberrations, or alterations to other regulatory mechanisms is needed to fully characterize the range of defects that lead to male infertility.

Epigenetic mechanisms, including DNA methylation, nuclear protein modification, and regulation by noncoding RNAs, influence male reproduction (2). Epigenomics therefore provides a promising approach to understand the etiology of some disorders, including unexplained male-factor infertility. Denomme et al. identified certain sperm histone epigenetic signatures that may predispose men with idiopathic male infertility to compromised blastocyst development during in vitro fertilization (IVF) (2). The sperm DNA methylation patterns and microRNA (miRNA) profiles identified by the authors highlight how aberrations in epigenetic regulatory networks may contribute to infertility in normozoospermic men. Adopting an epigenetic lens enables new insights into known infertility risk factors, such as obesity. Craig et al. overviewed how adiposity-induced alterations in the sperm epigenome at conception can be directly transmitted to offspring and increase the risk of metabolic derangements in progeny (3). These epigenetic investigations have deepened our understanding of the importance of weight control before conception and are helping to create new approaches to improve conception rates.

Although insights into epigenetic mechanisms from semen samples are important, the ability to further investigate these findings directly within the testes are hampered by the challenges associated with sampling testicular tissue. Interest in identifying blood-based epigenetic markers that complement biomarkers within the local testicular environment has increased. Factors including reactive oxygen species and environmental toxins can affect spermatogenesis by disrupting the hypothalamic-pituitary-gonadal axis. These androgenic axis disruptors may first induce epigenetic changes in the blood, and the blood-testis barrier may prevent immediately identifiable alterations in the testicular epigenome.

Friemel et al. pioneered work in this area by comparing DNA methylation profiles in peripheral blood samples between men with idiopathic infertility and fertile control men (4). Through this approach, they identified 471 CpG sites across 287 genes that were differentially methylated between the study groups. Gene ontology analysis of these differentially methylated regions highlighted enrichment of genes corresponding to major histocompatibility (MHC) class II receptor activity and piwi-interacting RNA (piRNA) binding. Furthermore 26 CpG sites corresponding to 15 genes were not imprinted and had been previously associated with fertility and spermatogenesis. These findings helped to elucidate proxy methylation markers for idiopathic infertility in peripheral blood and candidate loci outside the testes. Despite the novelty of this work, critical questions remained. Would these results be reproducible across different populations? Would other candidate genes for male infertility emerge via this approach?

The current work by Sarkar et al. builds on those findings by examining differential methylation of peripheral blood DNA in azoospermic and oligozoospermic infertile men compared with normozoospermic fertile control men (5). The authors examined methylation patterns with the use of a 450K Beadchip array, followed by deep sequencing of selected regions for confirmatory methylation analysis in the neighborhood regions of differentially methylated CpGs. Ultimately, the authors reported correlations between peripheral blood DNA methylation signatures and genes associated with male infertility. Of the 170 differentially methylated genes, at least 38 had already been implicated in spermatogenesis. Several targets identified via gene ontology analysis also corresponded to MHC class II receptor activity that had been previously identified by Friemel et al. The overlap between these two studies is exciting given that MHC class II complexes influence fertility, spermatogenesis, and pregnancy loss.

Many questions arise in light of these findings. Will peripheral blood–based epigenetic tests remain robust and cost-effective at scale? What are the optimal protocols to acquire samples for this assay? Where does epigenetic testing of peripheral blood samples reside in the spectrum of clinical testing for male infertility? Is such a test appropriate for all infertile men or only those with idiopathic infertility? Answers to these questions will help to further validate the clinical utility of this diagnostic approach and determine how this assay complements existing semen analysis and sperm function tests. Findings such as the ones presented by Friemel et al. and Sarkar et al. should motivate other investigators to help further develop this novel approach to profiling men with idiopathic infertility, and may lead to identification of additional targets for future therapies.

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