A NON-INVASIVE, EPIGENETIC-BASED SEMEN TEST TO PREDICT THE SUCCESS OF TESTICULAR SPERM EXTRACTION PROCEDURES

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Background: With 50% of mTESE procedures failing, can we develop a non-invasive, cost-effective, and highly sensitive assay to predict the presence or absence of sperm in the testicles?

Objective: Affecting 1% of men worldwide, NOA is the total absence of sperm in the ejaculate, and is the most severe form of male-factor infertility. Currently, men with NOA have markedly decreased chances of conceiving a child of their own. Despite recent progress in reproductive medicine, men with NOA have essentially one invasive and expensive procedure to isolate sperm (microdissection testicular sperm extraction or mTESE). Unfortunately, mTESE has a ~50% failure rate. Here we present the early development of an innovative DNA methylation-based technology to non-invasively predict the presence of sperm in the testicles of men with NOA.

Materials and Methods: From three clinical sites, testicular biopsies (N=14) and azoospermic semen samples (N=9) were procured from consented men who had undergone a testicular aspirate, biopsy or mTESE procedure. Utilizing new technology from Inherent Biosciences and Brigham Young University the biopsies and semen samples were sequenced and analyzed for sperm-specific DNA (Barney et al. 2022). Specifically, these samples were analyzed for a sequence of DNA that can distinguish sperm cells from all other somatic cells via a unique methylation pattern. Our hypothesis is the amount of sperm-specific DNA in the biopsies and semen can be used to determine the sperm in the testicles. The percent of sperm-specific DNA in the testicle and cell-free sperm-specific DNA in the semen were compared to the sperm extraction results.

Result(s): We found a statistically significant difference between the percentage of sperm-derived DNA in biopsies with no sperm identified compared to biopsies where sperm was identified ($p=0.001$). Sperm-derived cell-free DNA was successfully isolated and sequenced from a range of azoospermic and normospermic semen samples (N=8), demonstrating the feasibility of the novel technique. In semen samples from NOA men, there was a statistically significant difference in the percentage of sperm-derived cell-free DNA in semen samples with no sperm identified compared to sperm that was identified in the sperm extraction procedure ($p=0.005$). Semen samples where sperm was identified in the extraction procedure had >50x more cell-free DNA in the sample compared to samples where no sperm was identified.

Conclusion(s) : In the analysis of testicular biopsies and NOA semen samples, the sperm-specific DNA methylation patterns were highly predictive of both the absence and presence of sperm in the testicles. With these data the next steps of our work will focus on continued analysis of sperm-specific cfDNA in seminal plasma to be utilized as a novel tool for the prediction of sperm extraction success. The ultimate goal will be to develop a non-invasive and cost-effective way to avoid unnecessary surgical procedures.

References:

Barney, Ryan et al. “Assessment of seminal cell-free DNA as a potential contaminate in studies of human sperm DNA methylation.” *Andrology* vol. 10,4 (2022): 702-709. doi:10.1111/andr.13163

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