

Infertility affects 8-15% of couples in the United States. A male factor may contribute to infertility in up to 50% of couples. The etiology of male factor infertility is complex and for many men (up to 45%), no cause is identified. This study aims to revisit the role of sperm chromatin in fertility and assess its potential as a biomarker of idiopathic male infertility. Specifically, in this proposal we aim to evaluate sperm-specific protamines and their modifications in the setting of both normal and altered fertility. Protamines are small, highly-basic proteins essential for proper compaction of paternal DNA. In humans, two forms of protamine are necessary for correct packaging of DNA and normal fertility: protamine 1 (P1) and protamine 2 (P2). Prior studies utilizing gel electrophoresis have suggested that maintenance of the P1:P2 ratio is critical for normal fertility and spermatogenesis, however, previously used methodologies are unable to resolve different protamine isoforms. Furthermore, in 2014 mouse protamines were found to bear a number of post-translational modifications (PTMs). The function of these newly identified PTMs is unknown, however, preliminary data suggests that they are important for normal fertility in the mouse. In the human, the full spectrum of protamine PTMs is not known. In this proposal, we aim to further explore the presence and significance of protamine isoforms and PTMs in the setting of both normal and abnormal fertility. We hypothesize that human protamines will bear a number of post-translational modifications and that protamine isoforms and PTMs will be similar among fertile men with normal sperm. We further hypothesize that protamine isoforms and a subset of PTMs will be altered in men with infertility and abnormal spermatogenesis. To test these hypotheses, we will utilize highly accurate and quantitative nano-liquid chromatography mass spectrometry to assess both protamine isoforms and modifications. To begin to understand the acquisition of newly identified protamine PTMs we will also generate modification-specific antibodies and determine their presence in human testicular samples at various stages of the seminiferous tubule epithelial cycle. These pilot experiments will provide an important first step in understanding the role of protamine isoforms and PTMs in fertility and will allow for future studies assessing functional significance and large-scale clinical investigation.