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Quantitative post-translational modifications of developing sperm cells

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Sperm production is so unique, that the formation of a male gamete is virtually unprecedented. Starting as a round cell within the testis, a series of mitotic and meiotic events take place to produce what is morphologically considered to be a spermatozoon. During this process, remodelling of the DNA occurs in such a manner that at the end of testicular development, a sperm cell is incapable of further transcription or translation. Yet testicular sperm cells have no capacity for fertilization. As such, they are unable to undergo any form of motility, nor are they able to recognize, or bind to, or fuse with an egg. Such attributes are only found within sperm cells after they have undergone a 7-day transit through a second organ known epididymis. Remarkably, the only manner by which spermatozoa gain functionality (ability for movement, recognizing and fertilizing the egg) is through post-translational modifications of existing proteins.

To enhance our understanding of how a sperm gains the capacity for motility and sperm-egg recognition, we have used a label-free, quantitative (MS-based), approach to compare sperm cells of early (immature) and latter (mature) epididymal origin. Both phospho-peptides and glycopeptides (sialic-acid containing only) were enriched separately using Titanium dioxide. Following nano-flow, reversed phase chromatography with direct injection into a mass spectrometer, the MS-survey scan was used in silico to create virtual "2D-maps" by plotting the elution profile of individual peptides against their corresponding mass:charge ratios, together with the ion count. For the first time, we are able to show significantly regulated phospho- and glyco-peptides occurring during epididymal development (N=6). Importantly, we have shown that Izumo1, a protein that is essential for sperm-egg fusion, becomes phosphorylated 7 times during epididymal development. Production of phospho-mimic Izumo1 and WT Izumo1 recombinant proteins have demonstrated that phosphorylation regulates protein-protein interactions. As such, Sperm Equatorial Segment Protein 1 appears to bind to WT Izumo1, but not phospho-mimic. Importantly, the SPESP1 knockout mice demonstrate defective Izumo1 location. In addition to Izumo1, several pertinent observations have been found, including phosphoryaltion of Dynein Intermediate chain, which occurs as soon as sperm motility is regulated and glycopeptide changes on the essential ADAM family of proteins. These findings have significantly advanced our understanding of the molecular mechanisms regulating sperm epididymal transit.

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