A PROTEOMIC ANALYSIS OF RAT CAPUT AND CAUDA SPERM USING USING DIFFERENCE IN 2D-GEL ELECTROPHORESIS

***M. A. Baker*, R. Witherdin, L. Hetherington, K. Cunningham-Smith, J. Aitken**

Biological Science, University of Newcastle, Callaghan, NSW, Australia

When testicular spermatozoa migrate to the epididymis they are still functionally incompetent. Having lost the cellular machinery to support gene transcription and protein translation, these cells acquire the gamut of biological functions needed to achieve fertilization under the influence of factors provided by the epididymal microenvironment. Although the biological changes exhibited by spermatozoa during epididymal transit have been well established, the molecular basis for these changes is still poorly understood. Difference in 2D Gel electrophoresis (DIGE) is a powerful new technology for comparing up to three different protein samples in the same 2D gel, thus eliminating the variation that occurs with more traditional proteomic approaches. Using a combination of this procedure and matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry, we have unambiguously identified 8 proteins that change significantly during epididymal maturation including a-enolase, hsp60, endoplasmin, phosphatidyl-ethanolamine binding protein, testis lipid binding protein and the b-subunit of the F1 ATPase. The nature of these changes (80 kDa mass shift and increase electronegative charge) suggested a series of phosphorylation events. In order to further characterize these changes, Western blot studies were conducted using anti-phosphoserine antibodies. This analysis revealed a dramatic increase in serine phosphorylation for two major proteins (54 and 73 kDa) during epididymal transit, one of which (54 kDa) and confirmed by MALDI-TOF analysis to be the b-subunit of the mitochondrial ATPase. The phosphorylation of this protein was associated with a 3-fold increase in the ATP content of epididymal spermatozoa as they pass from the caput to the cauda epididymis. This change clearly identifies mitochondrial ATP production as a key component of the epididymal changes that lead to the generation of vigorously motile functional spermatozoa. The kinase responsible for the F1-ATPase phosphorylation appears to be PKA regulated and a clear potential target for contraceptive intervention.

10.1071/SRB04Ab209