## 65. LOCALISATION OF TYROSINE PHOSPHORYLATED PROTEINS ON MOUSE SPERMATOZOA DURING ZONA PELLUCIDA INTERACTION AND CHARACTERISATION OF SPERM SURFACE PHOSPHOPROTEINS

<u>Kelly L. Asquith</u>, Eileen A. McLaughlin, Brett Nixon and R. John Aitken ARC Centre of Excellence for Biotechnology & Development, Reproductive Science Group, University of Newcastle, NSW, Australia

Prior to fertilisation, mammalian spermatozoa undergo a series of molecular and biochemical events collectively termed capacitation. Although phosphorylation of sperm proteins on tyrosine residues has been recognised as an important correlate of this process, the precise relationship between protein phosphorylation and fertilizing ability has not previously been appreciated. This study demonstrates a direct association between tyrosine phosphorylation and zona pellucida binding affinity of mouse spermatozoa. Tyrosine phosphoproteins were localised to internal flagellar targets on 30% of capacitated spermatozoa and to plasma membrane head antigens on approximately 10% of capacitated cells. Further investigation revealed that almost all spermatozoa bound to the zona pellucida displayed phosphorylation of both head and flagellar proteins, suggesting that it is this sub-population of cells that are functionally competent. While these data implicate tyrosine phosphorylated sperm proteins in gamete interaction, our findings suggest that the zona pellucida recognition epitope itself is not phosphorylated. Rather, we propose that the sperm surface receptor may be a multimeric complex encompassing a zona pellucida recognition region and one or more tyrosine phosphoproteins. Activation of the complex by phosphorylation during capacitation may trigger changes in the sperm surface architecture that facilitate the formation of a functional zona receptor complex and allow recognition and adhesion to the oocyte. The current data imply an important role for phosphorylation as a post-translational mechanism of regulation that may be instrumental in the acquisition of sperm fertilizing ability. Phosphoproteome analysis of murine spermatozoa has revealed the identities of several proteins that may play an active role gamete interaction. Current research is focussed on elucidating the precise function of these candidates. These results contribute to our understanding of mammalian fertilisation and may provide insight into the fields of contraception and male infertility.