

## OOLEMMAL PROTEOMICS: IDENTIFICATION OF OOCYTE CELL SURFACE PROTEIN COMPLEXES INVOLVED IN SPERM-EGG INTERACTION

J. W. Paul<sup>1</sup>, E. A. McLaughlin<sup>1,2</sup>, R. J. Aitken<sup>1,2</sup>

<sup>1</sup>Reproductive Science Group, School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia; <sup>2</sup>ARC Centre of Excellence in Biotechnology & Development, Australia

While the molecular basis of sperm-oolemma interaction is of considerable biological significance, the protein(s) involved in this process are yet to be identified. In particular, the point at which developing mammalian oocytes acquire the capacity to bind to, and fuse with, capacitated, acrosome-reacted spermatozoa is yet to be clearly defined. Previous reports suggested that mature metaphase II (MII) mouse oocytes possessed the capacity to bind and fuse with spermatozoa while, in contrast, immature germinal vesicle (GV) phase oocytes did not. In this study oocytes were cultured in  $\alpha$ -MEM containing 5% fetal calf serum. For GV oocytes,  $\alpha$ -MEM was also supplemented with 1  $\mu$ M Milrinone to prevent GV breakdown. Standard murine IVF was performed in 100  $\mu$ L  $\alpha$ -MEM media droplets containing 10 to 15 oocytes and  $2.5 \times 10^4$  capacitated spermatozoa.

These studies found that no significant difference existed in the levels of sperm-oocyte binding or fusion between freshly isolated GV oocytes (14 sperm/egg bound, 7 sperm/egg fused), cultured arrested GV oocytes (16 sperm/egg bound, 7 sperm/egg fused) or cultured MII phase oocytes (17 sperm/egg bound, 8 sperm/egg fused). Interestingly, upon fusion MII oocytes commenced sperm nuclear decondensation whereas arrested GV oocytes did not.

Comparison of biotinylated oolemmal surface proteins revealed markedly different protein profiles between the GV oolemma and the MII oolemma. The MII oolemma revealed a multitude of proteins ranging in size from approximately 20 to 200 kDa, whilst the GV oolemma revealed only six middle range molecular weight proteins ranging from approximately 50 to 90 kDa. The protein(s) implicated in sperm-egg interaction must be one or more of those proteins found in both oocyte populations. Comparison of these two profiles has greatly reduced the number of possible candidates, allowing possible identification of the proposed GPI-linked sperm receptor.