25. CHARACTERIZATION OF THE RAT OOLEMMA

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In vitro manipulation of the murine embryo has advanced over the last 50 years with the introduction of ICSI, knockout and cloning technologies. Yet the same technologies have not developed in the rat despite high similarities between proteins with a function in fertilization and fundamental differences seem apparent. A robust *in vitro* culture medium, mR1ECM capable of supporting pre-implantation development through to the blastocyst stage has only recently emerged. *In vitro* culture (IVC) of *in vivo* fertilized embryos in mR1ECM with PVA showed 28.6 % of zygotes and 79.3 % of 2-cell embryos could develop to blastocyst. The major problem was in the initial division after collection. Almost half the failure to develop occurred at this point. KSOM proved to be significantly worse than mR1ECM+PVA for IVC with arrest prominent at the 2-cell stage (89.8%). mR1ECM even supported limited hatching of blastocysts from the zona pellucida (ZP). *In* vitro fertilization (IVF) with mR1ECM has not been successful. This may be due to failure of sperm to undergo capacitation. We unsuccessfully attempted to induce capacitation over a range (0.5–6.0 h) of preincubation times. A major difficulty is that a characterised marker for capacitation in the rat has not been identified. Another problem for successful IVF is that the majority of oocytes collected underwent spontaneous activation resulting in arrest and an oocyte incapable of fertilization (78.6%). This was characterised by cortical granule exocytosis and an alteration to the ZP molecular structure, which precludes sperm binding to the ZP. These significant limitations need to be overcome if we are to be able to study interaction between rat sperm and egg at the molecular level. The overall goal of this project is to be able to identify and characterise oolemma proteins that interact with sperm counterparts as the two gametes undergo fusion.

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