CULTURE OF MOUSE MALE GERM CELLS FOR GENETIC MANIPULATIONS

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The ability to maintain germ cells in culture offers the opportunity to manipulate their differentiation and to deliver new genes through the germline. However the study of male germ cell biology is hindered by the lack of an *in vitro* culture system that supports germ cell maintenance and differentiation, and the lack of efficient gene transfer technologies. To address this, we developed a short term (1-6 day) *in vitro* germ cell-Sertoli cell co-culture system using testes of 6 day old mice, in which the only germ cell type present is the spermatogonium. To establish culture conditions favourable for maintaining germ cells in culture, we compared different substrates (plastic, laminin, poly-L-lysine, Matrigel) and cell culture additives (insulin/transferrin, retinoic acid, lactic acid, pyruvic acid). Using immunocytochemistry, we observed that germ cell numbers varied depending on the substrate, and was greatly improved on laminin or Matrigel. The culture additives tested had no effect. We noted a striking difference in germ cell growth depending on the mouse strain used, with C57BlXCBA F1 cross > Swiss > BalbC >> C57Bl/6J. To investigate gene transfer into germ cells, constructs were generated using promoters identified as exhibiting germ cell-specific expression in transgenic animals (EF1-α, Oct4, Stra8) with EGFP and lacz reporter genes. From this baseline, we have started gene transfer experiments. Initial transfection experiments were performed in mouse testis cell lines (GC1, GC2, TM3, TM4) and the P19 mouse embryonal carcinoma cell line. Several commercial transfection agents and a non-commercial product (1) were used, as was electroporation, however these did not yield reporter expression in germ cells. As these techniques require proliferating cells, we are now undertaking a study with the addition of growth factors in culture (BMP4, activin, GDNF, FSH) to enhance germ cell proliferation and therefore improve transfection efficiencies. We will also employ lentiviral transduction, as this is reportedly 100% efficient and independent of proliferation.

(1) Celebi *et al*. (2002) *Mol. Reprod. Dev.* **62**,477–482.

10.1071/SRB04Ab213