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Long-Term Culture and Transplantation of Murine Testicular Germ Cells

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The objectives of this study were to develop an in vitro culture system to optimize germ cell proliferation and to measure the potential of the cultured germ cells to produce mature spermatozoa after transplantation into a recipient. Donor germ cells isolated from ROSA26 male mice were cultured with a STO feeder cell layer in Dulbecco's minimal essential medium (DMEM) supplemented with fetal bovine serum (FBS), stem cell factor, leukemia inhibitory factor, basic fibroblast growth factor, insulin-like growth factor 1, interleukin-11, L-glutamine, sodium pyruvate, 2-mercaptoethanol, murine oncostatin M, and platelet-derived growth factor. Donor germ cells formed colonies in the primary cultures after 8-21 days. These cultured colonies were maintained for 4 weeks or longer without subculture and proliferated for up to 8 passages over a period of 3 months. These colonies had alkaline phosphatase activity and incorporated 5-bromo-2'-deoxyuridine. These colonies were positive partially when screened with antibody for germ cell nuclear antigen and c-kit. Germ cells cultured with this supplemented medium showed enhanced colonization vs controls cultured with DMEM and FBS. Cultured germ cells from Rosa26 donors were transplanted into testes and were identified by X-gal staining and histological screening. The cells cultured in the supplemented medium colonized the tubules and initiated spermatogenesis in the recipient mice. This is an improved method for culturing germ cells and may be useful in gene therapy and the production of transgenic animals.

Key words: ROSA26 mice, germ cells, in vitro culture, testis, GCNA1, spermatozoa

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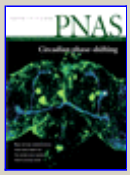

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