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Generation and Characterization of Male Germ Stem Cells from Mouse Embryonic Stem Cells to Evaluate Reproductive Toxicity of Toxic Chemicals

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Testicular germ cell tumors (TGCT) are the most common cancers in men aged between 15 and 44 years and the incidences have increased during last few decades. Association between TGCT and occupational exposure has been reported for agricultural workers, construction workers, paper, plastic and metal industry workers as well as in individuals exposed to PCBs and pesticides. However, the results are yet largely inconclusive and additional pre-clinical and epidemiological studies are much needed. *In-vivo* animal models have been used successfully to assess the testicular toxicity of several compounds however, these assays are time consuming and expensive. *In-vitro* models have been established however, the poor viability of freshly isolated germ cells has largely limited their use. Hence, the generation and development of a stable germ stem cell-line is of great interest. We report here the generation and characterization of a mouse male germ stem cell line from mouse embryonic stem cells (Celprogen Cat# 66101-28) using Celprogen's chemically defined media (Celprogen Cat# M66101-28S) and ECM (Celprogen Cat# E66101-28, 6-well plates). We also report here, the use of this cell based assay to evaluate testicular toxicity of environmental pollutants and toxic chemicals. Immuno-histochemistry and RT-PCR profiles of terminally differentiated mouse germ stem cells were performed to validate the new cell based assay. Expression profile of SSEA-1, c-Kit, OCT 3/4, DAZL, FABP9, VASA and Alkaline Phosphatase genes demonstrate the successful development of stable male mouse germ stem cells. *In-vitro* screening of chemical carcinogens, environmental pollutants and anti-cancer agents for their potential reproductive toxicity was performed using the newly developed cell based assay. The findings demonstrate high potential to utilize the male mouse germ stem cells and the procedure for the monitoring human exposure of male reproductive toxicants to establish exposure limit in the work environment.