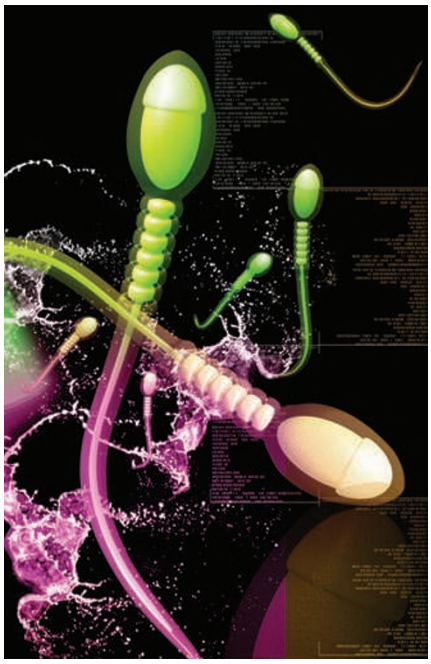
MALE FACTOR INFERTILITY

Milestone in autotransplantation of human spermatogonial stem cells

Functional human spermatogonial stem cells have been cultured and propagated long term for the first time, report Ans van Pelt and colleagues in the *Journal of the American Medical Association*. This impressive achievement should pave the way for recovery of fertility in survivors of childhood malignancy.

Infertility is an unfortunate adverse effect of cancer treatment in young boys. Unlike adults, from whom ejaculated sperm can be collected for cryopreservation, there is currently no means of restoring the fertility of male survivors of prepubertal cancer. van Pelt *et al*. hypothesized that cryopreserved testicular tissue collected before chemotherapy could be used as a source of spermatogonial stem cells for repopulation of the testes in adulthood. This autotransplantation technique has proved successful in several nonhuman species, including monkeys. Modification of this technique for application to young cancer survivors requires generation of a large number of stem cells from a small initial population. "Since only a small biopsy can be taken from a prepubertal testis, the number of stem cells [harvested] ... will not be enough to colonize a complete adult testis" explains van Pelt. "For that reason, it is essential to establish a culture system that allows for propagation of spermatogonial stem cells."



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The researchers cryopreserved testis tissue donated by 6 men with prostate cancer who had undergone orchiectomy. Testicular cells were isolated from thawed samples (mean recovery rate 80%) and cultured for up to 15 weeks in supplemented StemPro medium. Clusters of germline stem cells and embryonic stem cell-like colonies were observed in cultures from all 6 men after a mean of 22.5 days; these were subcultured, and could be propagated for up to 28 weeks. Spermatogonial RNA and proteins were detected throughout the culture period, and differentiation into cells from all 3 germ layers was noted.

Functionality was tested at several stages of *in vitro* propagation via xenotransplantation of cells into the testes of immunodeficient mice. Detection of human-specific *COT-1* using fluorescence *in situ* hybridization confirmed colonization of mouse seminiferous tubules by viable spermatogonial stem cells from 4 of 6 donors. The number of stem cells increased by a factor of 50 during a 19-day stretch early in the culture period, and more than 18,000-fold during a 2-month period later in the cycle.

At these growth rates, a stem cell population large enough to recolonize an adult testis could be generated from a small amount of prepubertal tissue. van Pelt's team reached this conclusion after adjusting for the 60-fold discrepancy in volume between an adult testis and a small testis biopsy (approximately 13 ml versus 200 µl) and the 20-fold increase required to account for the low efficiency of transplantation.

Clinical demand for this new tool is likely to be high. About 1 in 250 young adults have survived childhood cancer, and more than 60% of the parents of children diagnosed with cancer currently elect to store a sample of their son's testis tissue before treatment.

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