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Journal of Andrology, Vol. 23, No. 5, September/October 2002 Copyright © American Society of Andrology

Development of an In Vivo Model to Study Testicular Morphogenesis

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We have developed an in vivo model to examine testicular cord formation by isolated Sertoli and myoid cells when implanted under the kidney capsule of severe combined immunodeficient (SCID) mice. Neonatal porcine Sertoli (92.5% \pm 3.5%) and myoid (2.2% \pm 0.7%) cellular aggregates were transplanted underneath the kidney capsule of SCID mice. Grafts were removed between 0 and 60 days posttransplantation and examined histologically for the progressive

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development of structures resembling testicular cords. Aggregates began to reorganize by day 3, and cord structures were present at day 7 posttransplantation. These structures became larger and more defined as the time progressed after implantation. To localize Sertoli and peritubular myoid cells, grafts were immunostained for the Sertoli cell proteins, vimentin, DNA transcription factor GATA-4, and Müllerian inhibiting substance (MIS), as well as for a myoid cell protein, smooth muscle alpha-actin. In the "seminiferous" epithelial layer, the Sertoli cells were arranged with their nuclei along the basal edge adjacent to the peritubular myoid cells that were surrounding the tubules. Moreover, the expression of MIS mimicked that during porcine testicular development, suggesting the Sertoli cells were developing normally. In addition, proliferating cell nuclear antigen (PCNA) was detected in the Sertoli cells at all time points, indicating the proliferation of Sertoli cells in the grafts, which is consistent with Sertoli cell proliferation prior to puberty in the native porcine testis. These results suggest that the specific factors required for cord formation and prepubertal development are inherent in the transplanted cells. Moreover, we have developed a novel in vivo transplantation model to study seminiferous cord formation and prepubertal development.

Key words: Sertoli cell, peritubular myoid cell, cord formation, severe combined immunodeficient mice, testis development

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