Journal of Andrology, Vol 17, Issue 6 708-717, Copyright © 1996 by The American Society of Andrology

JOURNAL ARTICLE

Isolation of highly purified type A spermatogonia from prepubertal rat testis

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We have developed a new method that allows isolation of highly purified type A spermatogonia from prepubertal rats. The procedure is based on the maximal release of spermatogonia from the seminiferous epithelium obtained by the complete enzymatic digestion of the tubular basal lamina, followed by removal of contaminating somatic cells through adhesion to plastic dishes coated with the lectin Datura stramonium agglutinin and fractionation on a discontinuous Percoll gradient. The cell suspension obtained contains up to 85% type A spermatogonia. Besides morphological criteria, the identification of germ cells and somatic cells has been performed by means of immunocytochemical markers, such as c-kit receptor, which is present only in germ cells, and vimentin, which is present only in somatic cells. All type A spermatogonia isolated were c-kit positive, thus suggesting that c-kit receptor is present in both undifferentiated and differentiating type A spermatogonia. Preliminary culture experiments demonstrate that spermatogonia survival in vitro was significantly improved by the addition of 10% fetal calf serum or horse serum to the culture medium; however, optimal culture conditions remain to be established. In vitro studies on isolated spermatogonia may provide a significant contribution toward elucidation of the mechanisms regulating spermatogonial proliferation and differentiation.

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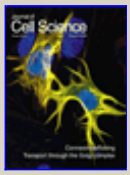

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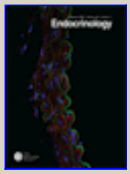

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