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JOURNAL ARTICLE

Flow cytometric DNA analysis of defined stages of rat seminiferous epithelial cycle during in vitro differentiation

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The stages of the rat seminiferous epithelial cycle have been isolated for flow cytometric analysis of DNA and for culture, using transillumination-assisted microdissection. Precise stages have been identified by phase contrast microscopy of live cell squashes from adjacent segments. Each stage of the cycle showed a characteristic flow cytometric pattern with haploid (1C), diploid (2C) and tetraploid (4C) peaks. Stages I to VIII of the cycle showed an additional hypofluorescent (0.25-0.70C) peak due to a reduced dye-binding capacity of maturation phase-spermatids at steps 15 through 19. The appearance of the hypofluorescent haploid peak coincided with the second nucleoprotein transition at step 15 of spermiogenesis and the homogeneous condensation of the chromatin seen in electron microscopy. As a concomitant of the formation of disulphide bonds during epididymal maturation, the fluorescence intensity decreased further to reach a relative value of 0.07C in the cauda epididymidis. The constant 1C peak was raised by round and elongating spermatids (steps 1-14), 2C by spermatogonia, secondary spermatocytes and Sertoli cells, and the 4C peak by primary spermatocytes and spermatogonia at G2 or M phase of the mitotic cycle. The proportion of each peak accurately reflected the relative proportion of cells in most stages of the cycle when compared with morphometric measurements of histologic preparations. DNA flow cytometry is a suitable method for quantitative evaluation of cultured seminiferous tubule segment DNA. Although the relative yield of the meiotic reductive divisions in vitro is comparable with that observed in vivo, steps 9 and 15 of spermiogenesis involving nucleoprotein transitions and spermiation itself did not occur under the present culture conditions.

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