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Gene Transfer to Mouse Testes by Electroporation and Its Influence on Spermatogenesis

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We transferred the adventitious gene pCAGGS-lacZ to mouse testes with the use of a square-wave electroporator and investigated the efficiency of gene transfer (GT) and the influence of the procedure on testicular damage and spermatogenesis. Mice were divided into 5 groups: (1-2) injection of gene/phosphate-buffered saline (PBS) into the interstitial space followed by electroporation (EP), (3) EP alone, (4-5) injection of gene/PBS without EP. The presence of the *lacZ* gene was determined by X-gal (5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside) staining and the polymerase chain reaction (PCR). The influence of transfer on spermatogenesis was assessed by evaluating the seminiferous tubules according to the Johnsen score (JS). TdT-mediated dUTP-biotin nick end-labeling (TUNEL) staining was performed for the detection of apoptosis in the testes to evaluate the testicular damage caused by GT, and fertilization ability was assessed by mating male mice from each group with normal female mice at 1, 2, 4, 6, and 8 weeks after the procedure. LacZ expression was detected by X-gal staining and PCR for 4 weeks after GT in group 1. But in group 4, LacZ expression was not detected for all times. In groups 1 through 3, the JSs decreased gradually until 4 weeks and recovered at 6 and 8 weeks after GT. The JSs were significantly decreased at 4 weeks for groups 1 through 3 compared with groups 4 and 5. In groups 1 through 3, apoptotic cells were significantly more numerous at 1, 2, and 4 weeks after the procedure, and there were significant differences in their numbers between groups 1 through 3 and groups 4 and 5 until 4 weeks after the procedure. The number of offspring did not differ significantly between all groups. These results suggest that although spermatogenic damage caused by EP could present problems, GT by EP might be effective for transfecting germ cells or somatic cells and could be applicable for in vivo gene therapy for male infertility in the future.

Key words: In vivo electroporation, *lacZ*, testis, apoptosis, gene transfection, ß-gal activity