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Identification of active spermatogenesis using a multiphoton microscope

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Abstract

Background: The sperm retrieval rate of microdissection testicular sperm extraction (Micro-TESE) varies from 25 % to 60 %, suggesting that the subjective decision of each operator affects the success of sperm retrieval outcomes. Therefore, it is necessary to establish an objective selection criteria for identifying seminiferous tubules with spermatozoa.

Objectives: Our aim was to develop a method for identifying spermatogenesis without sectioning of testicular tissues.

Materials and Methods: Testicular tissues of 10-week-old normal rats were fixed with 4 % paraformaldehyde. Fluorescent labeling of seminiferous tubule nuclei and F-actin was performed, and the specimens were observed without sectioning using a multiphoton microscope. Cryptorchid rats were used as a model lacking elongated spermatids. Multiphoton images were compared with images of normal seminiferous tubules. In addition, seminiferous tubules of 10-week-old normal rats were labeled by testicular interstitial injection of fluorescent probes and observed by a multiphoton microscope without fixation. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-stained images of normal and probe-injected testes were compared.

Results: In fixed seminiferous tubules, elongated spermatids were identified. In addition,

F-actin of apical ectoplasmic specialization (apical ES) were observed around elongated spermatids. Furthermore, spermatogenic stages were identified by an array of nuclei or F-actin. In cryptorchid testes, elongated spermatids and F-actin of the apical ES were not observed. In testes injected with fluorescent probes, F-actin of the apical ES were observed, and spermatogenic stages were identified without fixation. There was no significant difference in the number of TUNEL-positive cells per seminiferous tubule between normal and probe-injected testes.

Conclusions: Seminiferous epithelium could be observed without sectioning of tissues by fluorescent probes and a multiphoton microscope. Active spermatogenesis was observed by labeling F-actin with and without fixation. Moreover, the toxicity of fluorescent probes was limited. Our method has a potential for live imaging of testicular tissue.