

Journal of Andrology, Vol 21, Issue 5 625-635, Copyright © 2000 by The American Society of Andrology

## JOURNAL ARTICLE

# Glycerol disrupts tight junction-associated actin microfilaments, occludin, and microtubules in Sertoli cells

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Intratesticular injections of glycerol have been shown to result in a marked and prolonged reduction of spermatogenesis, accompanied by increased permeability of the blood-testis barrier. Because the permeability of the blood-testis barrier is regulated by Sertoli cell tight junctions, and tight junction organization is regulated by the cytoskeleton, we undertook to examine the effects of glycerol treatment on cytoskeletal actin microfilaments and microtubules, and on the tight junction protein, occludin, in Sertoli cells. Adult rats received a single intratesticular injection of either saline (controls) or a 10% glycerol solution. At 24 hours and 7, 15, and 21 days after injection, testes were collected and prepared for routine histology, cryosectioning, or whole seminiferous tubule immunohistochemical staining; and the preparations were viewed by light and confocal microscopy. In saline-injected testes, Sertoli cells had a cytoskeletal and junctional organization that resembled that of normal testes. F-actin microfilaments, located in the basal region, were arranged in regular bundles or chords that circumscribed the perimeter of each Sertoli cell at the level of the tight junction. Occludin colocalized with tight junction-associated actin filament distribution and microtubules formed a geometric array associated with spermatogenic cells. In contrast, in glycerol-treated Sertoli cells, microfilament and microtubule organization and occludin distribution were partially or completely disrupted. From these results we conclude that glycerol treatment either directly or indirectly disrupts tight junction-associated F-actin and occludin and tubulin organization in rat Sertoli cells. Perturbation of the tight junction-associated proteins could explain the increase in permeability of the blood-testis barrier observed after glycerol treatment. Impaired spermatogenesis following glycerol treatment is likely a consequence of a leaky blood testis barrier and disrupted Sertoli cell cytoskeleton. Glycerol injections may serve as a useful tool in studying the relationship between cytoskeletal organization and the stabilization of Sertoli-Sertoli cell junctions.

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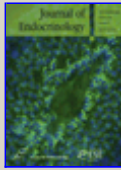
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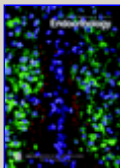
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