

## IDENTIFICATION OF ELEVATED LEVELS OF APOPTOSIS AMONG T-CELLS ISOLATED FROM THE RAT TESTIS

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Protection of the developing gametes from attack by the immune system is essential for reproductive success. Autoimmune infertility represents a failure of this protection. Specific T-cell apoptosis is the main mechanism for control of antigen-specific immune responses. Studies were undertaken to investigate this regulatory process in adult rat testes. Flow cytometry was employed in conjunction with annexin-V/propidium iodide dual staining to identify apoptotic cells concurrent with CD3 staining to identify T-cells. CD3-positive cells isolated from the testicular interstitial tissue were shown to be  $34.12 \pm 3.0\%$  apoptotic (mean  $\pm$  s.e.m.,  $n = 3$ ) at collection. This was consistently greater than the numbers of apoptotic CD3-positive cells isolated from lymph nodes ( $4.04 \pm 1.95\%$ ,  $n = 2$ ), spleen ( $16.77 \pm 4.73\%$ ,  $n = 4$ ) and peripheral blood ( $9.64 \pm 1.44\%$ ,  $n = 2$ ). These results also were confirmed by using T-cells purified with MACS microbeads against the pan T-cell marker OX52 to improve sample purity: 40% of isolated testicular T-cells and 3% lymph node T-cells were found to be undergoing apoptosis. The level of apoptosis among T-cells isolated from another non-lymphoid organ, the liver, was only 6%. It is hypothesised that the immunosuppressive milieu of the testis induces an increased level of apoptotic deletion among T-cells that gain entry into the testis and potentially threaten gamete viability. Further studies of the mechanism responsible for this elevated level of T-cell apoptosis in the testis will significantly enhance our knowledge of how testicular immune tolerance is maintained.