During mammalian spermatogenesis, 90%-99% of histones are removed from germ-cell chromatin and replaced by protamines, but the location of retained histones and mechanisms keeping them there are poorly understood. The authors examined chromatin accessibility during mouse spermatogenesis using ATAC-seq and found retention at promoters, inter- and intragenic regions, and repetitive elements. Linking histone hyperacetylation to chromatin reorganization, inactivation of the histone acetyltransferase Gcn5 in sperm cells caused increased sperm histone retention and defective male fertility. Abnormal histone retention and histone acetylation could prevent normal nucleosome eviction during spermatogenesis and underlie certain male fertility defects.