OBJECTIVES: Female sex workers (FSWs) form a core group at high risk of both sexual HIV acquisition and secondary transmission. The magnitude of these risks may vary by sexual risk taking, partner HIV prevalence, host immune factors and genital co-infections. We examined temporal trends in HIV prevalence and per-act incidence, adjusted for behavioral and other variables, in FSWs from Nairobi, Kenya. METHODS: An open cohort of FSWs followed since 1985. Behavioral and clinical data were collected six monthly from 1985 to 2005, and sexually transmitted infection (STI) diagnostics and HIV serology performed. A Cox proportional hazards model with time-dependent covariables was used to estimate infection risk as a function of calendar time. RESULTS: HIV prevalence in new FSW enrollees peaked at 81% in 1986, and was consistently below 50% after 1997. Initially uninfected FSWs remained at high risk of acquiring HIV throughout the study period, but the rate of HIV acquisition during unprotected sex with a casual client declined by over four-fold. This reduction correlated closely with decreases in gonorrhea prevalence, and predated reductions in the Kenyan HIV population prevalence by over a decade. CONCLUSIONS: The per-act rate of HIV acquisition in high-risk Nairobi FSWs fell dramatically between 1985 and 2005. This decline may represent the impact of improved STI prevention/therapy, immunogenetic shifts in at-risk women, or changes in the proportion of HIV exposures occurring with clients who had acute HIV infection. Declining HIV incidence in high-risk cohorts may predict and/or be causally related to future reductions in population prevalence.