

ABSTRACT

Variable infectivity and transmissibility of HIV/SHIV has been recently associated with the menstrual cycle, with particular susceptibility observed during the luteal phase in non-human primate models and *ex vivo* human explant cultures, but the mechanism is poorly understood. Here, we performed an unbiased, mass spectrometry-based proteomic analysis to better understand the mucosal immunological processes underpinning this observed susceptibility to HIV infection. Cervicovaginal lavage samples (n=19) were collected, characterized as follicular or luteal phase using days since last menstrual period, and analyzed by tandem-mass spectrometry. Biological insights from these data were gained using a spectrum of computational methods including hierarchical clustering, pathway analysis, gene set enrichment analysis, and partial least-squares discriminant analysis with LASSO feature selection. Of the 384 proteins identified, 43 were differentially abundant between phases ($p < 0.05$, $\times 2$ fold change). Cell-cell adhesion proteins and antiproteases were reduced, and leukocyte recruitment (IL-8 pathway, $p = 1.41E-5$) and extravasation proteins ($p = 5.62E-4$) were elevated during the luteal phase. LASSO/PLSDA identified a minimal profile of 18 proteins that best distinguished the luteal phase. This profile included cytoskeletal elements and proteases known to be involved in cellular movement. Gene set enrichment analysis associated CD4⁺ T cell and neutrophil gene set signatures with the luteal phase ($p < 0.05$). Taken all together, our findings indicate a strong association between proteins involved in tissue remodeling and leukocyte infiltration with the luteal phase, which may represent potential hormone-associated mechanisms of increased susceptibility to HIV.

Importance Recent studies have discovered an enhanced susceptibility to HIV infection during the progesterone-dominant luteal phase of the menstrual cycle. Yet, the mechanism responsible for this enhanced susceptibility has yet to be determined. Understanding the source of this vulnerability will be important for designing efficacious HIV prevention technologies for women. Furthermore, these findings may also be extrapolated to better understand the impact of exogenous hormone application, such as the use of hormonal contraceptives, on HIV acquisition risk. Hormonal contraceptives are the most widely used contraceptive method in sub-Saharan Africa, the most HIV burdened area of the world. For this reason, research conducted to better understand how hormones impact host immunity and susceptibility factors important for HIV infection is a global health priority.