Recapitulating human menopausal vaginal tissue with an ovariectomized mouse model

Gisele A. Calderon[1], Jennifer M. McCracken[1], Quynh N Le[1], Natasha M Faruqui[1], Swathi Balaji[2], Sundeep G. Keswani[2], Julie C-E Hakim[1,2]

[1]Baylor College of Medicine and Texas Children's Hospital, Department of Obstetrics and Gynecology, Division of Pediatric and Adolescent Gynecology, Houston, Texas, USA

[2] Baylor College of Medicine and Texas Children's Hospital, Department of Surgery, Division of Pediatric Surgery, Houston, Texas, USA

Abstract

All women experience menopause in their lifetime due to vastly reduced estrogen associated with aging. Women can also experience hypoestrogenic states by other means such as treatment against pelvic cancers as well as genetic and eating disorders. The resulting vaginal environment fosters debilitating vaginal symptoms like atrophy, dryness, and dyspareunia. Current treatment options, like estrogen and hyaluronan (HA), anecdotally improve symptoms, but rectifying mechanisms are largely understudied. **In order to study these changes in estrogen states, understand the mechanisms behind current treatments, and develop new therapies, we developed and characterized a reliable and reproducible animal model for evaluating hypoestrogenic vaginal tissue**. We performed bilateral ovariectomies (OVX) on 9-week-old CD1 mice using naïve and sham-operated mice as controls. By harvesting the reproductive tract after one month, we could characterize the phenotype that is associated with human vaginal tissue in an estrogen reduced state. Developing new treatments for hypoestrogenic vaginal symptoms rely on better understanding the altered cellular and tissue environment. Through this baseline characterization report, further studies using this mouse model has potential to advance women's vaginal health treatments.

Approach

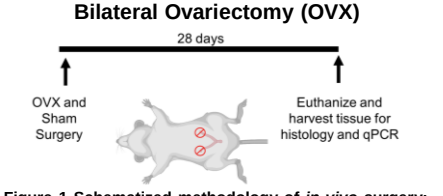

Figure 1 Schematized methodology of *in vivo* surgery: Depiction of our surgical approach and timeline between the removal of the murine ovaries (or sham surgery) to the analytical end point after 28 days of recovery.

Results

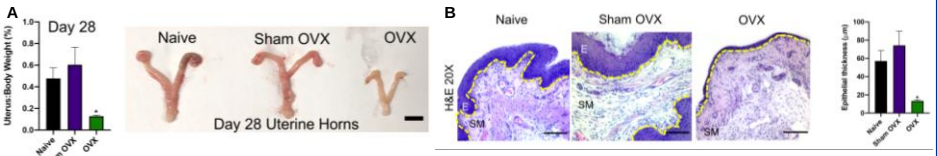

Figure 2 Estrogen absent milieu alters vaginal tissue: Ovariectomized mice exhibit arrested circulating estrogen leading to altered phenotypes where (A) the full reproductive tract shrinks and results in a significantly reduced uterine to body weight ratio. Scale bar, ~5 mm. (B) Moreover, the OVX vaginal epithelial layer significantly thins. Additionally, highly rugated epithelium is a hallmark characteristic of healthy vaginal tissue. However, with reduced estrogen, we observe thinned and flattened epithelium in the OVX condition. Scale bar, 100 μm.

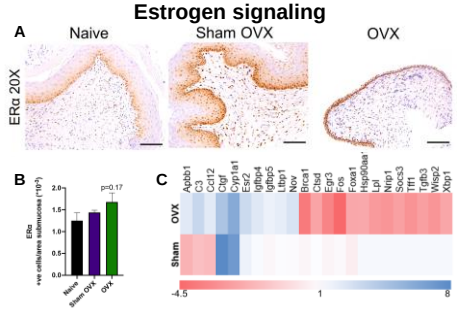

Figure 3 Altered estrogen signaling in OVX: IHC expression of ERα reveals differential localization across the epithelium the highest density concentrated at the epithelium's basal side across all groups. Because OVX produces a thin epithelial layer, ERα appears ubiquitously within that layer of the epithelium. (b) However, the submucosa possesses greater ERα density in OVX. (c) The shifted response in expression occurs in some upregulated genes but mostly downregulated genes in the reduced estrogen state associated with OVX. All scale bars, 100 μm.

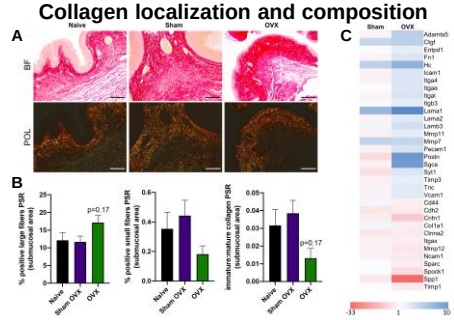

Figure 4 Disruption in ECM and collagen profiles for a vaginal atrophic state. (A) Picrosirius red (PSR) representative images. B) By assessing percent area covered by corresponding colorimetric parameters of red (large, type I collagen) and yellow/green (small, type III collagen) fibers, we showed OVX vaginal tissue sections demonstrated trends toward an increase in large fibers and a decrease in small fibers. C) We also demonstrated that a low-estrogen environment dysregulates ECM gene transcription. All scale bars, 100 μm.

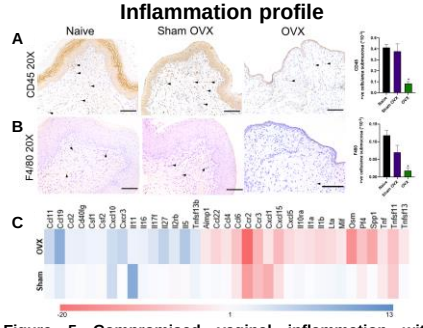

Figure 5 Compromised vaginal inflammation with reduced estrogen. In vaginal tissue sections, CD45$^+$ and F4/80$^+$ cells were significantly reduced in OVX mice, compared to sham and naïve, by immunohistochemistry (A) and (B) thus potentially reducing the tissue's capacity to respond to potential insult. C) Estrogen dependent chemotactic and inflammatory gene profiles were altered in OVX versus sham-operated mice. All scale bars, 100 μm..

Conclusion

We have evaluated a bilateral ovariectomy mouse model to study estrogen/ECM and immune alterations in vaginal tissue. This model recapitulates the phenotypic features observed in human reduced estrogen states and found an associated decrease in basal vaginal inflammation. We propose this could lead to lower defense mechanisms that often make postmenopausal women victims of vaginal infections.

References

Calderon GA*, McCracken JM*, Le QN, Faruqui N, Hakim JE. An ovariectomized murine model recapitulates the altered vaginal environment in an estrogen reduced state. *Under revision*. | Funding: NIGMS 1K08GM135638-01.