The neutral vaginal pH in mice that is typical of most mammalian species should not deter research using experimental murine models of Candida vaginitis

Commentary: Exogenous reproductive hormones nor Candida albicans colonization alter the near neutral mouse vaginal pH

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Abstract

For over three decades, investigators have used estrogen-dependent rodent animal models to study pathogenesis of vulvovaginal candidiasis (VVC) or test promising antifungal drugs. One disparity not well publicized is that rodents maintain near neutral vaginal pH, which is in contrast to the acidic vaginal pH in women. In this issue of Infect. Immun., Peters and co-workers have addressed the topic with an elegant study that not only confirms the near neutral vaginal pH in mice, but also reveals a stable vaginal pH that is not influenced by exogenous reproductive hormones or *C. albicans* vaginal colonization (1) (IAI00550-20). More importantly, they make a convincing argument that the neutral vaginal pH should not deter using the model for research purposes. This commentary further emphasizes the points made and attempts to provide a more global perspective on this interesting property of the animal model.
Vulvovaginal candidiasis is an extremely common mucosal disease, caused primarily by *Candida albicans* and characterized by itching, burning, pain, and redness of the vulva and vaginal mucosa often accompanied by vaginal discharge (2). It is estimated that 75% of otherwise healthy immunocompetent women of childbearing age will experience primary (episodic) VVC at least once in their lifetime (3). Although treatment of primary VVC with antifungals is usually successful, approximately 5-8% of afflicted women will suffer from recurrent VVC (RVVC), characterized by four or more symptomatic episodes per year often requiring continual (maintenance) antifungal therapy (2). Predisposing factors for primary VVC include high-estrogen oral contraceptive use, hormone replacement therapy, antibiotic usage, uncontrolled diabetes mellitus, and disruption of the vaginal microbiota (3-6). RVVC is considered idiopathic with no identified predisposing factors, although the mechanisms of VVC and RVVC pathogenesis are likely identical (2, 7). Importantly, VVC and RVVC are not associated with immunodeficiency, but are instead associated with a vigorous local immunopathogenic inflammatory response. Much of the work that led to the mechanisms of immunopathogenesis and drug efficacy were conducted using the estrogen-dependent rodent models of VVC.

Interestingly, despite ongoing investigations using rodent animal models (mouse, rat) (8) for more than three decades, the property of a near neutral vaginal pH in the rodents, in contrast to the acidic vaginal pH in humans, was never a direct focus of investigation. Apart from this disparity, the estrogen-dependent rodent models closely parallel the acute and chronic nature of the disease in women. Accordingly, the animal models have been a valuable tool to dissect the host response and test antifungal drugs.
to obtain information that is translatable to the human disease. There are several reasons for the lack of primary references reporting a neutral vaginal pH in rodents. First, the property relative to experimental VVC was noted more in methodological papers, book chapters describing the models, or relevant review articles on the models (8-10). Second, the pH was never the true focus of a primary publication; it never adequately fit with antifungal drug studies sponsored by pharma/industry or pathogenesis/host defense studies. Third, the model simulated the clinical disease so well that the vaginal pH was never considered a confounding factor (11, 12). Hence, this disparate property never seemed critical enough to convey/discuss explicitly in the general modes of scientific communication. However, now that Peters and co-workers have published a study that comprehensively focuses on vaginal pH in mice as it relates to experimental VVC, it seems timely to emphasize the significance, or lack thereof, of the vaginal pH relative to past and future experimentation using the experimental murine VVC model.

More interesting than acknowledging the seemingly atypical neutral vaginal pH in rodents is the fact that although an acidic vaginal pH is relatively common knowledge when speaking of women of childbearing age, humans are one of the few mammalian species that harbor an acidic vaginal pH (13, 14). As Peters and co-workers point out, most other mammalian species harbor a near neutral vaginal pH likely due to low levels of commensal *Lactobacillus* species that are abundantly present in the human vaginal lumen (15). Hence, this would preclude establishing alternative animal models for VVC studies if an acidic vaginal pH were a strict requirement. But as noted earlier, the experimental mouse model of VVC simulates clinical VVC despite this environmental
In light of this information, the acidic vaginal pH in humans should actually be considered atypical. Another point to be emphasized is that the near neutral vaginal pH provides a permissive environment for hyphal formation by inoculated *C. albicans*, which is a property required for virulence and pathogenicity (16). This is true for almost all anatomical sites in the experimental models of candidiasis, including the vaginal cavity (17). In fact, the only experimental model of candidiasis that does not require the transition from yeast to hyphae by *C. albicans* is the intra-abdominal infection (IAI) model of fungal/bacterial sepsis (*C. albicans/S. aureus*) (18). In more recent studies, the production of candidalysin by *C. albicans* during hyphal formation has been shown to be critical for the vaginal immunopathogenic response through its ability to trigger invasion of the vaginal mucosa (19). This extremely permissive environment for hyphal formation/candidalysin production provided by the neutral vaginal pH makes for a very robust vaginal infection model. So much so that we often point out that if antifungal drugs show efficacy in the robust mouse model, there is a high likelihood that efficacy will be achieved clinically. Similarly, if positive results are observed when testing immunotherapeutics or tools to dissect pathogenicity, there is a strong likelihood for biological significance. An interesting question that is often asked is why the rodent vaginal cavity does not harbor *C. albicans* in any morphological form if so permissive to growth and pathogenesis. Intuitively, based on the widespread environmental presence of *C. albicans*, one would expect a relatively high prevalence of *C. albicans* and the potential for acute VVC in rodents and other mammalian species. However, all inbred and outbred commercial sources of rodents (both mice and rats) show no detectable
natural colonization of *C. albicans* or other *Candida* species in the vaginal tract. Nothing is known about other wild mammals, although there is no indication of widespread VVC among other mammalian species despite the neutral vaginal pH. One advantage of the lack of endogenous *Candida* species in rodents for researchers is that it provides a naïve host model for effective experimentation.

Another interesting question from a clinical perspective related to neutral vaginal pH and *Candida* hyphal formation is why isn’t VVC a common co-infection during cases of bacterial vaginosis (BV), which is characterized by an increase in vaginal pH? It would seem the BV-associated vaginal environment accompanied by a neutral pH would be permissive to *C. albicans* hyphal formation, and subsequently those women harboring *C. albicans* as an asymptomatic commensal might be expected to present with VVC. However, VVC and BV co-infections are rare (20). It is possible that the change in the vaginal environment by the reduced lactobacilli or presence of BV pathogens is not permissive to *C. albicans* growth despite the increase in vaginal pH.

These interesting caveats may be as simple as recognizing, as Peters and co-workers note, that vaginal pH is actually not a major driving force clinically for the presence or absence of VVC/RVVC. The vaginal pH is not impacted by an episode of acute VVC or RVVC, and as noted above, the higher vaginal pH during BV does not result in increased prevalence of VVC. This can be said of the mouse model as well via data presented by Peters and co-authors. Despite the permissive pH environment for hyphal formation/virulence, the presence of *C. albicans* or the state of pseudoestrus, a fundamental component of VVC animal models, has no impact on the vaginal pH which remained consistently near neutral in several mouse strains of distinct genetic
backgrounds. Additionally, the vaginal pH of CD-1 mice, an estrogen-hyporesponsive 
vaginitis-resistant strain (21, 22), remains neutral during the short period of colonization 
post-inoculation prior to rapid clearance. Thus, resistance and susceptibility to VVC in 
mouse strains is independent of vaginal pH. This together with the documented 
similarities with the clinical immunopathogenesis of VVC, including the neutrophilic 
infiltration/inflammatory local environment/neutrophil anergy/dysfunction relative to anti-
_Candida_ activity (23), supports the concept that the pH issue should not deter use of the 
model for future antifungal drug studies or pathogenesis research. The only exception 
would be for investigators who desire to test pH-dependent _Candida_ mutants or 
environments where an acidic pH is required. This was clearly evident in past studies in 
which interpretations of data were based on the assumption of an acidic vaginal 
environment that was not experimentally confirmed and likely was neutral, thereby 
confounding the results (24). If indeed an artificial acidification of the murine vaginal 
environment is desirable, one possible strategy would be through exogenous vaginal 
colonization with _Lactobacillus_ species (25, 26). However, it should be noted that 
vaginal pH was not evaluated/monitored following colonization.

In conclusion, despite the inherent vaginal pH disparity between mice and 
humans, rodents have and will continue to represent strong clinically relevant animal 
models for the study of VVC. But now that Peters and co-workers have elevated the 
vaginal pH issue by this comprehensive study highlighting this previously understated 
phenomena, investigators should have all the necessary evidence to make informed 
decisions regarding use of the models for their research.
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