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Case Study

A Win for Women's Health: EpiVaginal Brings Human-Relevant Safety Testing to Feminine Products

In March 2026, the FDA released draft guidance to assist sponsors in implementing streamlined approaches for nonclinical safety assessments to replace or reduce animal toxicology studies with New Approach Methodologies (NAMs) "hoping to receive appropriate NAMs data in place of animal data when available." After decades of animal testing as the unquestioned default, the regulatory ground is shifting. The work described in this case study is one example of scientific collaboration involving Mattek's (now part of Sartorius) lab-engineered human tissue models, Johns Hopkins University, and Good Clean Love that helped drive the field to this point.

The Problem with the Standard

Every year, millions of women use feminine care products trusting that they've been rigorously tested and proven safe. But for decades, the test meant to provide that assurance was never conducted on anything close to human tissue.

Personal lubricants are classified as Class II medical devices by the U.S. Food and Drug Administration (FDA). That status requires manufacturers to conduct a battery of biocompatibility tests before a product can go to market. The standard battery, as defined by the FDA's Center for Devices and Radiological Health (CDRH), includes an *in vivo* rabbit vaginal irritation (RVI) test, a guinea pig maximization skin sensitization test, an acute systemic toxicity test in mice or rabbits, and an *in vitro* cytotoxicity assay on flat cell monolayers (Costin *et al.*, 2020).

None of these test systems closely resemble the actual vaginal environment. Consequently, many products cleared by these standard tests have been found to pose risks to human health. Research shows that the majority of widely available vaginal lubricants are formulated with osmolality levels four to twenty three times higher than that of healthy vaginal fluid (Ayehunie *et al.*, 2018). Multiple studies have linked hyper-osmolal formulations to disrupted vaginal barrier function, increased risk of bacterial vaginosis, and higher susceptibility to sexually transmitted infections including HIV.

The testing system meant to protect women was, in many ways, failing them.

Why Animal Models Are Falling Short

Animal-based safety testing became established because regulators needed whole-organism responses to potential toxicants. For decades, the RVI test was considered the gold standard for evaluating vaginal irritation. The limitations of these models have become increasingly difficult to ignore, however.

The rabbit vagina and the human vagina are very different. Two-thirds of the rabbit vaginal lining consists of columnar epithelium, whereas the human vaginal epithelium is a multilayered stratified squamous structure eight to twelve cells thick. Rabbits also lack the cyclic hormonal stages, vaginal Lactobacilli, lactic acid acidity, and cervical mucus production that define the human vaginal microenvironment (Costin *et al.,* 2020).

Critically, the rabbit vagina also serves as a urethra, routinely exposing it to the highly variable osmolality of urine. This means rabbits are naturally desensitized to osmolality changes that are harmful to human tissue. A product that is damaging to the human vaginal epithelium may not show a notable response in the rabbit model (Ayehunie *et al.,* 2018).

A Human Tissue Model Built for Human Biology

Mattek Life Sciences developed the EpiVaginal tissue model to address exactly the gaps that animal models leave open.

Built from normal, human-derived vaginal-ectocervical epithelial cells, EpiVaginal is a three-dimensional, highly differentiated tissue that closely recapitulates the structure and physiology of *in vivo* vaginal tissue. Cells are cultured on microporous membrane inserts at the air–liquid interface, producing a multilayered epithelium complete with glycogenfilled suprabasal layers, the hallmark feature of healthy human vaginal tissue.

In addition to the stratified architecture, the model has the barrier properties and cellular differentiation of actual vaginal mucosa. It can be used in its full-thickness form (VEC-FT), which includes a fibroblast-containing lamina propria and supports epithelial-stromal cross-talk, or in a partial-thickness format (VEC-PT) optimized for high-sensitivity screening (Figure 1).

Beyond safety testing, the same biological fidelity makes EpiVaginal a platform for HIV and STI transmission research, microbicide development, and drug delivery evaluation. It further supports investigation of how hormonal changes influence vaginal physiology and infection susceptibility.

Hormone-Responsive and Physiologically Relevant

One of the most compelling demonstrations of EpiVaginal's biological fidelity is its responsiveness to reproductive hormones. Research by Ayehunie *et al.* (2015) showed that the tissue model expresses both estrogen receptors (ER-α and ER-β) and progesterone receptors in patterns that closely parallel *ex vivo* tissue. When treated with estradiol, the tissues increased in thickness, showed enhanced barrier function as measured by transepithelial electrical resistance (TEER), and accumulated glycogen in the suprabasal layers, all consistent with the proliferative phase of the human menstrual cycle. Progesterone treatment produced the opposite effect: epithelial thinning and reduced barrier integrity, mimicking the secretory phase.

Estradiol treatment also upregulated a broad range of immune-related gene pathways, including interferon-induced genes, interleukin signaling, and wound-healing responses, biological processes directly relevant to vaginal susceptibility to infection. These are responses that standard animal models or flat cell cultures cannot replicate.

Reproducible Across Labs and Over Time

For any test method to earn regulatory confidence, it must be reliable. A multi-site reproducibility study was presented at the 2024 European Society of Toxicology *In Vitro* (Klausner *et al* 2024). It compared EpiVaginal tissue performance across three independent laboratories in the United States, Slovakia, and the Czech Republic. All 31 tissue lots produced during the 2024 study met the quality acceptance criteria established in 2006-2007, a span of nearly two decades of consistency. All 31 tissue lots produced during 2024 met the quality acceptance criteria established in 2006–2007, a span of nearly two decades of consistency.

Intra-lot variability, measured by coefficient of variation, remained below 10% across all sites. When testing the same set of five feminine care products, the two independent testing labs produced results within an average of 10.1% for tissue viability (MTT assay) and 9.0% for barrier integrity (TEER).

The robustness can be reinforced by the recently published Reis *et al.* 2025 study in Toxicology Letters (P18-89 Bridging Physiological Accuracy and Worldwide Reproducibility: This level of reproducibility across labs and over nearly twenty years of tissue production is a meaningful differentiator from animal-based models, which are known for high inter-lab and inter-animal variability.

In Action: Detecting Harm That Animal Tests Miss

The most direct demonstration of EpiVaginal's advantage over the RVI test came from a collaboration between Mattek, Johns Hopkins University, and Good Clean Love, a manufacturer of organic feminine care products. Researchers tested nine commercially available vaginal lubricants on the EpiVaginal model, comparing iso-osmolal and hypo-osmolal formulations against hyper-osmolal products (Ayehunie *et al.,* 2018).

First, the team established that healthy vaginal fluid has an osmolality of approximately 370 ± 40 mOsm/Kg. Against that baseline, the hyper-osmolal lubricants tested ranged from 1,500 to 8,600 mOsm/Kg, four to twenty-three times the osmolality of the vaginal environment.

The EpiVaginal model clearly discriminated between these product groups. Lubricants with osmolality greater than four times that of vaginal fluid caused progressive, measurable disruption to the basal and parabasal cell layers of the tissue, confirmed by both TEER measurements and histological scoring. TEER values fell by 30–70% for the most hyper-osmolal products. The histological damage ranged from disorganization of the parabasal layers to complete separation of the epithelial layers from the membrane support.

Iso-osmolal and hypo-osmolal products, including Good Clean Love formulations, showed essentially no detectable damage to the basal or parabasal layers.

Notably, the standard MTT viability assay, the only *in vitro* test currently included in the regulatory biocompatibility battery, failed to detect this damage. The clinical relevance of this finding is significant. This finding is clinically significant because it demonstrates that products passing current regulatory testing may still compromise the protective function of women's vaginal tissue.

Advancing the Regulatory Pathway

The scientific evidence generated with EpiVaginal has been central to a broader regulatory push to replace the RVI test.

In 2016, a consortium including the Institute for *In Vitro* Sciences (IIVS), the PETA International Science Consortium, and the Consumer Healthcare Products Association, with participation from major personal lubricant manufacturers, submitted a proposal to the FDA to qualify an *in vitro* vaginal irritation test through the Medical Device Development Tools program. FDA reviewed the proposal and send back comments but did not accept it. However, based on the Good Clean Love request, FDA is accepting cytotoxicity data on case-by-case basis until it is fully validated and qualified. Under the program, IIVS is pairing EpiVaginal data with historical RVI data to build a prediction model that can be submitted in regulatory applications in place of the animal test (Costin *et al.,* 2020). Because CDRH is the only regulatory agency worldwide that routinely requires the RVI test for personal lubricants, a successful qualification would effectively eliminate it globally.

More recently, the FDA Modernization Act 2.0 and the European Union's ban on animal testing for cosmetic ingredients have reinforced this momentum, as has the FDA's March 2026 draft guidance on New Approach methodologies (NAMs), which signals a consistent agency-wide direction toward human-relevant testing.

A Better Standard of Safety

The gap between what current regulatory testing requires and what it actually detects has real consequences for the health of women who use these products every day.

Mattek is helping to close gaps in regulatory toxicology testing with a wide range of lab-engineered human tissue models that are scientifically rigorous, human-relevant, and reproducible alternatives to traditional models. The combination of biological fidelity, validated reproducibility across independent laboratories, and an active regulatory qualification pathway positions EpiVaginal as the leading platform for the next generation of vaginal safety assessments.

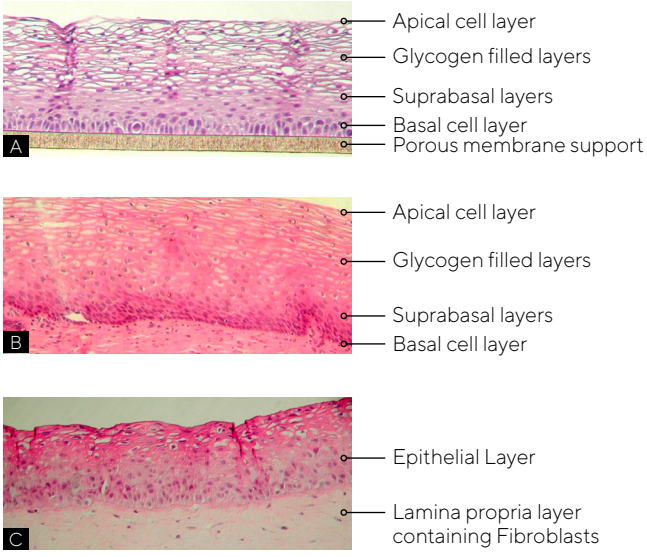

Figure 1: Histological cross-section of A) EpiVaginal, and *in vitro* reconstructed human vaginal epithelium, B) human vaginal explant tissue, C) EpiVaginal-FT Tissue, an *in vitro* reconstructed human vaginal full-thickness tissue model with fibroblasts.

For manufacturers, it means the ability to screen formulations with confidence before they reach regulators. For researchers, it means a physiologically relevant window into the vaginal microenvironment. And for the women who ultimately use these products, it means a safety standard that is finally, and unambiguously, built around their biology.

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Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

For further information, visit
sartorius.com