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HIGH RESOLUTION IMAGING OF EPITHELIAL INJURY IN THE SHEEP CERVICOVAGINAL TRACT:

A PROMISING MODEL FOR TESTING SAFETY OF CANDIDATE MICROBICIDES

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Abstract

Background—Access to readily available large animal models and sensitive non-invasive techniques that can be used for the evaluation of microbicide-induced changes in tissue could significantly facilitate pre-clinical evaluations of microbicide safety. The sheep cervicovaginal tract, with stratified squamous epithelium similar to humans, holds promise as a large animal model used prior to non-human primates. In addition, optical coherence tomography (OCT) could enable high resolution visualization of tissue morphology and non-invasive assessment of microbicide-induced epithelial injury.

Methods—We evaluated the dose response of sheep cervicovaginal tract to benzalkonium chloride (BZK). Twenty sheep received treatment with phosphate buffered saline (PBS) or BZK solution (2%, 0.2%, or 0.02%). Pre- and post-treatment colposcopy and OCT images were collected and graded based on WHO criteria and a previously reported scoring system, respectively. Biopsies were collected and the degree of epithelial injury and its thickness was assessed based on histology and OCT.

Results—The sheep cervicovagina exhibited anatomical and microscopic features similar to the human. Extensive loss of the epithelium was noted on colposcopy and OCT following application of 2% BZK. Colposcopy detected findings in half of sheep and OCT in all sheep treated with 0.2% BZK. OCT detected differences in the 0.02% BZK-treated group compared to controls whereas colposcopy failed to detect any changes.

Conclusions—The sheep cervicovagina is similar to humans, and exhibits dose dependent epithelial changes following BZK treatment. These findings suggest that the sheep model and OCT may become valuable tools for the safety evaluation of candidate microbicides, and warrant continued development.

Short summary—Optical coherence tomography, a high resolution imaging method, used with colposcopy in the sheep cervicovaginal tract detected a dose-dependent response to benzalkonium chloride, demonstrating potential for microbicide safety evaluations.

Keywords

optical coherence tomography; microbicides; vaginal product safety; sheep; cervix

INTRODUCTION

Microbicides will be used by healthy women to prevent HIV and sexually transmitted infections (STI's); therefore these products require an exceptional safety profile. The nonoxynol-9 (N-9) experience demonstrates the complexity of assessing microbicide safety (1). N-9 has been used as a spermicide for many years and has been believed to be safe. However, in two clinical trials testing the ability of N-9 to prevent HIV and other STI's, investigators found an increase in the incidence of HIV infection compared to placebo recipients (2,3). Similarly, a recent trial of cellulose sulfate was halted due to concerns about safety (4). Preclinical and early clinical studies failed to identify potential toxic effects and did not indicate that there may be issues with safe use of these products (5-8). Thus, there is a need to develop new methods that can better evaluate the safety and toxicity of candidate microbicides.

Current animal models used in pre-clinical safety assessment studies include small animals such as mice and rabbits and non-human primates (NHP). Small animals have been extensively used for initial evaluation and screening of microbicide safety (9-12) and offer the advantage of being readily available and low cost. However, one concern with these models is that the epithelium is columnar (10) which is different than human stratified squamous epithelium. The cervicovaginal tract in NHP exhibits similar anatomy, epithelial morphology and thickness, and vaginal flora to humans. Consequently NHP are believed to be suitable for the evaluation of microbicide safety (13,14); however, NHP are expensive and there are limited numbers available. Furthermore, they require special housing and extensive training and experience for handlers. In addition, the ability to obtain biopsies to evaluate microbicide-induced microscopic changes in epithelial tissue is limited. Thus, there is a need for a large animal model that is readily available and can be used as an intermediate model in which products could be screened and prioritized for evaluation in the NHP model. In the studies described here we evaluated the sheep as a model for microbicide safety evaluation. Sheep are relatively inexpensive, docile, easy to handle and examine, and available in large numbers with the ability to control for age, reproductive history, and sexual history.

To date assessment of the effects of microbicides on the integrity of the cervicovaginal epithelium has relied primarily on colposcopic examination and biopsy of NHP and humans. Unfortunately colposcopy is insensitive to detect minute microbicide-induced changes in the integrity of the epithelial layer and biopsy is invasive (1,6). Colposcopy allows the gross

appearance of the cervix and vagina to be determined based on white light reflectance imaging; however, it has not been a reliable predictor of safety in clinical trials (6,8). The mechanism of injury that leads to increased susceptibility is believed in part to be due to epithelial disruption or thinning (15-17), changes that cannot be consistently visualized with colposcopy. A method that can measure both epithelial thickness and examine for loss of the epithelium has potential to be more sensitive to find subtle or early injury caused by topical application of microbicides. Thus, there is a need for the development and utilization of a more sensitive imaging system that can non-invasively assess the morphology of cervicovaginal epithelium.

Optical coherence tomography (OCT) is a noninvasive imaging method which can be used to complement colposcopy to evaluate microbicide effects in the cervix and vagina (17,18) by providing high resolution (10-20 μm), cross-sectional imaging of tissue microstructure. OCT gives information on tissue morphological structure and enables in vivo measurement of the epithelial layer thickness (18,20). It is used routinely by ophthalmologists to examine morphological features of the retina (21) in the clinical setting. Because OCT is non-invasive it allows repeated evaluations of the same tissue over time for comparisons of baseline and post-treatment findings. Preclinical studies suggest that the high-resolution afforded by OCT has potential to detect toxicities not detected by colposcopy (18). In a recent pilot study conducted in a NHP model, we demonstrated that OCT images could be classified and scored allowing non-invasive quantification of the microbicide-induced changes in epithelial morphology and thickness (18). In the current study, we examined the response of sheep cervicovaginal epithelial tissue to increasing concentrations of benzalkonium chloride (BZK) using colposcopy, OCT imaging, and histological findings.

MATERIALS AND METHODS

Animals

Twenty female virginal yearling Rambouillet sheep (25-35 kg) were used in this study. All procedures were approved by the IACUC of the University of Texas Medical Branch, Galveston, Texas.

Procedure

Anesthesia was initiated with intramuscular and intravenous ketamine, xylazine, and diazepam. Intubation was performed and isoflurane was administered throughout the procedure. The animals were placed dorsal supine on a V-tilt table.

A pediatric Graves speculum was used to visualize the vagina and cervix. After baseline imaging was performed with colposcopy and OCT, the animals were awakened and extubated. Sheep received 8.0 ml of a solution containing 2% BZK ($n = 2$), 0.2% BZK ($n = 5$), 0.02% BZK ($n = 5$), or PBS ($n = 8$) intravaginally using an atraumatic ball-tip catheter. Twenty-four hours later, the sheep were intubated and evaluated by colposcopy, OCT, and biopsy. After imaging, the sheep were sacrificed and the complete reproductive tract, including the vulva, vagina, cervix, uterus, tubes, and ovaries removed. All groups were treated with a single dose except the sheep in the 2.0% BZK group, which received four daily doses. This group had colposcopy and OCT obtained 24 hours after the first and fourth doses, and biopsy obtained 24 hours after the fourth dose. The length of the vagina was measured in 6 control sheep. In six sheep, pH measurements were obtained before imaging using standard pH test strips. Excised cervices and vaginas from 4 untreated sheep were also evaluated with histology from biopsy for comparison to treated animals.

Imaging

Colposcopy—An Olympus (Center Valley, PA) colposcope with digital camera attachment was used to assess the cervix and vagina by white light low power magnification (7.5-12X). An experienced colposcopist graded findings during the exam following the WHO/CONRAD guidelines for colposcopic evaluation of vaginal products (22). Findings with both intact epithelium and vasculature included erythema, grossly white finding, blanching, and edema. Findings with disrupted vasculature included petechiae and ecchymosis. Disrupted epithelium was classified as superficial or deep disruption. Superficial peeling or abrasion was considered superficial disruption. Bleeding or disruption into the sub-epithelial tissue was considered deep disruption. Colposcopic observations of the cervix and vagina were noted on daily examination records, and documented by digital photography.

Optical Coherence Tomography—After conventional colposcopy, OCT imaging was performed as previously described (18). OCT images of the vagina and cervix were obtained at predetermined sites as well as at colposcopically abnormal areas of the cervix and vagina. Images were obtained from four separate regions of the cervix and vagina, with three images collected at each site, for a total of twenty-four images of the cervix and vagina. Cross-sectional OCT images were generated and graded by an experienced masked grader using a modification of a previously reported scoring system developed during NHP studies (18). Category 1 images had epithelium present, category 2 images had partial loss of the epithelium, and category 3 images exhibited full loss of the epithelium. Where epithelium was present on vaginal OCT images, the thickness was measured using Presto 32 software (Optimec Ltd/BioMedTech LLC, Nizhny Novgorod, Russia), with 3 epithelial thickness measurements taken per image.

Histology

Punch biopsies were obtained at the site of each OCT image 24 hours after treatment, with 4 biopsies each of the cervix and vagina. Hematoxylin and eosin (H&E) stains were made and evaluated for epithelial integrity and degree of inflammation in a blinded manner by a pathologist. In addition, the number of epithelial cell layers was counted and vaginal epithelial thickness was measured in six locations on each vaginal biopsy using Spot Advanced software (Diagnostic Instruments, Sterling Heights, MI).

Data Analysis

OCT scores and epithelial thickness were analyzed using mixed model for repeated measures. Multiple comparisons were used to compare (a) mean OCT scores at the cervix and vagina by treatment group at baseline and 24 hours after treatment and (b) mean epithelial thickness measurements by treatment group. Since the outcome measurements for OCT scores were ordinal (1 to 3), categorical analysis (frequency table and Cochran-Mantel-Haenszel Statistics) was also used to verify the results from mixed model analysis. The results from the categorical analysis were consistent with those from the mixed analysis, so only results from the mixed model analysis are presented. All the computations were conducted using SAS system (SAS/STAT: *User's Guide, Version 9*; Cary, NC: SAS Institute, 2002).

RESULTS

Sheep Cervicovaginal Model Results

Sheep anatomy is similar to humans in a number of ways, including anatomic dimensions and microanatomical structure (Table 1) (23-27). It was feasible to use a vaginal speculum for visualization of the cervix and vagina with colposcopy in the sheep. Colposcopy and OCT images of sheep were similar to humans (19,28,29) (Figure 1a-d, Figure 2a-c). On histology evaluation from biopsy, the sheep vaginal and cervical epithelium consisted of stratified

squamous epithelium overlying dense vascular submucosa, thinner than but similar in structure to human epithelium (23,26). Figure 2g shows cervical tissue after a single dose of PBS, which is typical of that seen in PBS-treated as well as untreated tissue.

Assessment of Tissue Response to BZK Treatment

Colposcopy Findings—Figure 1 shows representative colposcopy images at baseline and after treatment, with study findings summarized in Table 2. At baseline, colposcopy showed the cervix and vagina to be pink, intact tissue, with petechiae and erythema seen in some animals in all treatment groups. These findings have also been reported in humans and macaques at baseline in microbicide studies (14,30,31). It was not unusual to see thin intact vessels throughout the vagina and cervix. Twenty-four hours after treatment, colposcopy findings of PBS-treated animals were comparable to findings at baseline. In contrast, in animals that received 2% BZK, colposcopic changes that included edema, blanching of the epithelium, and peeling were seen. In the animals that received 0.2% BZK, there were variable colposcopy findings with three sheep exhibiting peeling and two sheep with pink, intact epithelium. These animals also had occasional petechiae and erythema. In the animals that received 0.02% BZK, no differences were seen on colposcopy as compared to control animals, with erythema and petechiae occasionally seen.

In the sheep, the vaginal sphincter, the narrowest portion of the vagina, was commonly the site of erythema, ecchymosis, and epithelial disruption attributable to speculum trauma; therefore these findings were not included in the table. This is consistent with human studies, in which colposcopy findings that are related to applicator or speculum trauma are noted as such.

During colposcopy, observations of the vaginal fluid were also made. Discharge in the untreated animals at baseline was typically minimal to moderate with mucus. In sheep treated with PBS, the discharge 24 hours after treatment was similar to baseline. In the 2.0% BZK treatment group, the exudate was profuse with thick sheets of tissue up to 1 cm in length. In the 0.2% BZK treatment group, the vaginal fluid had “sloughing tissue” or tissue debris. In the group treated with 0.02% BZK, no peeling or disruption was seen on the cervix or vagina, however, the discharge contained small clumps of tissue-like debris.

OCT Findings—At baseline, OCT images were similar for animals in all groups, with a normal layered structure revealing intact epithelium (Figure 2). Twenty-four hours after a single treatment with PBS, vaginal and cervical OCT images were comparable to baseline. Vaginal OCT images 24 hours after 0.02% BZK treatment were also similar to baseline. However, OCT images from the cervix of 0.02% BZK treated animals showed partial loss of the epithelial layer (Figure 3). After treatment with 0.2% BZK, OCT showed that the vaginal and cervical epithelium was largely disrupted with only small areas where it was intact (Figure 2). For 2.0% BZK treated sheep, the OCT images showed overwhelming loss of the epithelial layer (not shown).

OCT Scores: Before treatment, OCT scores in each treatment group were comparable ($p=0.25$ vagina, $p=0.47$ cervix). After treatment, scores were increased in all BZK treatment groups when compared to PBS. Baseline and twenty-four hour post-treatment scores were similar in the sheep treated with PBS (Figures 4 and 5). In the group treated with 0.02% BZK, OCT image scores were significantly higher than the PBS control group ($p=0.0004$) in the cervix (Figure 4) but similar to PBS control scores ($p=0.96$) in the vagina (Figure 5). In the group that received 0.2% BZK, the OCT scores for the cervix and vagina were significantly higher than that of PBS ($p<0.0001$), indicating loss of the epithelium. In the 2% BZK group, the OCT scores were also significantly higher ($p<0.0001$) for the cervix and vagina than that of the PBS control animals, reflecting loss of the epithelium.

OCT thickness measurements: Vaginal epithelial thickness was measured from OCT images at baseline and 24 hours after treatment. In PBS treated sheep the mean ($\pm$ SE) epithelial thickness at baseline was 94 ± 9 μ m and did not change significantly after treatment at 91 ± 5 μ m. Similarly, sheep treated with 0.02% BZK were 89 ± 11 μ m at baseline and 86 ± 6 μ m after treatment. In contrast, treatment with either 0.2% BZK (91 ± 11 μ m to 16 ± 6 μ m) or 2.0% BZK (90 ± 17 μ m to 6 ± 10 μ m) significantly reduced epithelial thickness ($p<0.001$ each).

Histology Findings—Figures 2 and 3 demonstrate histology findings after treatment with PBS, 0.02% BZK, and 0.2% BZK. After treatment with PBS, epithelium was intact with leukocytes in the epithelium and submucosa. After treatment with 0.02% BZK, differences were noted when compared to the PBS-treated group. Epithelium was present in most biopsy sections; however the cervix had diffuse subtle changes in the epithelium including loss of epithelial cells and separation the cell layers by edema. The vagina exhibited less frequent changes including aggregates of leukocytes, loss of epithelial cells, and intercellular edema. After treatment with 0.2% BZK, large areas of the epithelium were thinned or fully lost, however some epithelium was still present in focal areas. Inflammatory infiltrate was present as well.

Epithelial Thickness Measured by Histology: Vaginal epithelial thickness was measured from biopsy samples collected 24 hours after treatment. In PBS treated animals the epithelium was 100 ± 7 μ m (Mean $\pm$ SE) which was comparable to measurements obtained by OCT. In the sheep treated with 0.2% BZK, the epithelium was significantly thinned (16 ± 8 μ m; $p<0.001$) and the measurements were again in good agreement with those obtained from OCT images. In animals treated with 0.02% BZK, the epithelium was 69 ± 8 μ m which was significantly reduced compared to controls ($p=0.01$) and less than the value obtained by OCT.

DISCUSSION

New methods for determining microbicide safety, including the development of new large animal models and sensitive non-invasive techniques to detect epithelial injury, are urgently needed. In the studies reported here we have shown that the sheep cervicovaginal tract exhibits gross and microscopic similarities to the human. In the course of these studies we used BZK, an agent known to produce vaginal epithelial disruption in macaques, pigs, and humans (14, 32,33), at a range of concentrations to assess the response of sheep cervicovaginal tissue using colposcopy and histology from biopsy while exploring the sensitivity of OCT imaging for in vivo detection of microbicide-induced epithelial injury.

NHP, while considered the gold standard for pre-clinical safety evaluations due to their similarity to humans, are expensive and the numbers of subjects and availability of biopsies is limited. Thus, there is a need for development of an intermediate animal model to be used in safety evaluations prior to NHP and clinical studies; the sheep holds promise as this new model. The sheep cervix and vagina were similar to humans when evaluated using colposcopy, OCT, and histopathology. In addition, sheep are docile, inexpensive, and easy to handle, and multiple biopsies can be obtained in a single animal. Sheep have been used for many years in perinatal health and development studies and are well characterized for these purposes (34).

Dose-related BZK-induced changes were noted in the sheep cervicovaginal tissue when evaluated by the imaging methods of colposcopy, OCT, and light microscopy of H&E stained biopsies. Baseline colposcopy findings of the cervix and vagina included intact epithelium with occasional erythema and petechiae. This is not unexpected given that baseline findings are also common in humans (31,35,36) and NHP (14,30). Colposcopy findings in the sheep were more severe for a single dose than that found in macaques at a similar concentration of BZK (14). There were findings of erythema (3/5 animals) and petechiae (1/5 animals) with a single dose

of 1.2% BZK in the macaque, however, after a single dose in the sheep, there was blanching and superficial disruption (peeling) in all sheep (n = 2) treated with 2% BZK and superficial disruption (peeling) in three out of five sheep treated with 0.2% BZK. After four daily doses of 1.2% BZK, three out of five macaques had vaginal disruption. Although macaques were treated with BZK multiple times, more severe epithelial injury was observed in the sheep cervicovaginal tissue. This suggests that the thinner epithelium in the sheep, measuring 86-114 μm , may be more sensitive for the detection of injury than that in the macaque, which measures 90-220 μm (37).

OCT imaging provides the ability to visualize superficial and sub-epithelial microbicide induced changes in tissue morphology and measurements of epithelial thickness. OCT detected dose-related changes in the sheep model after treatment with BZK. OCT scores increased with increasing concentrations of BZK, with greater loss of epithelial integrity with increasing doses; higher OCT scores indicated greater levels of injury to the tissue. These findings were consistent with injury and loss of the epithelium seen on biopsy.

Epithelial thickness is an important parameter however it cannot be measured by any current safety evaluation method except for biopsy. It is believed that thinning or loss of the epithelial barrier is a factor in increasing the risk of infection with HIV (15,16). In NHP, animals with thinned epithelium after progesterone treatment were more susceptible to SHIV infection (16) and conversely, those treated with estrogen to thicken the epithelium were protected (38). As demonstrated in the current study, OCT can be used to measure epithelial thickness non-invasively before and after treatment. It could also be used repeatedly over the course of a longer study to track epithelial thickness and assess the health of epithelium as a function of time following topical applications of microbicides. Dose related changes were detected with OCT measurements of epithelial thickness, which decreased with increasing doses of BZK, a finding consistent with that from the OCT scores. The 0.2% and 2.0% groups had significantly thinner epithelium after treatment than at baseline. Epithelial thickness measured from biopsy also confirmed these findings, with thinner epithelium in the 0.02% and 0.2% BZK groups when compared to the PBS group. While many of the comparisons reached statistical significance, we recognize the limitation in this study of performing statistical comparisons on small groups of animals.

OCT vaginal thickness measurements were thicker than histology measurements in the 0.02% BZK group by approximately 17 micrometers, or about 1-2 cell layers. A contributing factor could be microscopic loss of the epithelium, with denuding of mildly injured superficial epithelial cells during biopsy acquisition and processing. Histology evaluation showed subtle changes in the existing epithelium including intercellular edema causing cellular separation. Furthermore, the colposcopy findings of vaginal fluid with debris in the absence of peeling in the 0.02% BZK group suggest that epithelial cells are being disrupted.

In the development of a model involving the female reproductive tract, the potential for cyclic changes due to hormones must be considered. In studies in mice, the cycle is synchronized with Depo-Provera to limit the variability of the cyclic hormonal effects on the vaginal epithelium, causing the epithelium to be suspended in a relatively thin state rather than undergoing rapidly changing vaginal thickness in the four day cycle (39). In humans and NHP, the general consensus has been that the 28 day menstrual cycle does not have a significant effect on vaginal tissue and its response to microbicide treatment; therefore the subjects are allowed to cycle naturally (13,26). Sheep have a seventeen day estrous cycle, so a study such as this one involving only 24 hours of evaluation would be expected to have a limited impact from cyclical hormonal tissue changes. Therefore in this study we did not artificially alter the natural cycle of the sheep.

In summary, the current study showed that the sheep cervicovaginal tract has similarities to humans that may make it useful for microbicide safety evaluation. Similar to the NHP model, the sheep cervicovaginal model exhibits dose dependent epithelial changes following BZK treatment as assessed by colposcopy, OCT, and biopsy. Overall, OCT scores increased and OCT epithelial thickness decreased with increasing doses of BZK, a finding supported by the decreased epithelial thickness with increasing doses measured with histology. The findings from this study suggest that both the sheep model and OCT warrant continued development.

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Figure 1.

Colposcopy findings in sheep cervix and vagina at baseline and twenty-four hours after a single intravaginal treatment with PBS or 0.02%, 0.2%, or 2% BZK. At baseline, erythema, petechiae, or no findings were detected (a-d). Twenty-four hours after treatment, the PBS (e) and 0.02% treated sheep (f) were similar to baseline. Superficial disruption (peeling) (arrow) is seen after treatment with 0.2% BZK (g). The sheep treated with 2% BZK had global blanching and thickening as well as peeling (arrow) (h).

Figure 2.

Representative OCT images of the vagina with corresponding histology. OCT images at baseline and at 24 hours after a single dose treatment with PBS, 0.02% BZK, or 0.2% BZK are shown with the corresponding H&E stained image from biopsy 24 hours after treatment. At baseline, all OCT images are similar, showing the layered structure of an intact epithelium with underlying submucosa (a, b, c). After treatment, the OCT image in the PBS-treated sheep was comparable to baseline (d). The corresponding biopsy confirmed intact epithelium (g). After treatment, with 0.02% BZK treatment the OCT image is also comparable to baseline (e). The corresponding biopsy reveals intact epithelium with evidence of subepithelial inflammation (h). After treatment with 0.2% BZK there was disrupted epithelium, detected both by OCT (f) and histology (i). (OCT image scale is in millimeters. H&E stain 40X magnification. Arrows denote boundary of epithelium and submucosa. ep, epithelium; s, submucosa; de, epithelial disruption).

Figure 3.

Representative OCT images of the cervix with corresponding histology. OCT images are shown with corresponding histology from biopsies 24 hours after a single dose treatment with PBS or 0.02% BZK. OCT images and histology images after PBS treatment show intact epithelium (a, c). In contrast, OCT and histology images after 0.02% BZK treatment show epithelium with areas of disruption (b, d). (OCT image scale is in millimeters. H&E stain 40X magnification. Arrows denote boundary of epithelium and submucosa. ep, epithelium; s, submucosa; de, epithelial disruption).

OCT Scores - Cervix

Figure 4.

OCT scores in the cervix at baseline and twenty-four hours after a single treatment with PBS (n=8) or 2% (n=2), 0.2% (n=5), or 0.02% (n=5) BZK. At baseline, scores were similar ($p=.47$). In the sheep treated with 2% and 0.2% BZK, scores were significantly higher after treatment than scores in the PBS-treated sheep ($p<0.0001$). In the sheep treated with 0.02% BZK, the scores at the cervix were significantly higher than that of PBS ($p=0.0004$). (Error bars indicate 95% confidence intervals. Asterisks indicate statistical significance.)

OCT Scores - Vagina

Figure 5.

OCT scores at baseline and twenty-four hours in the vagina after a single treatment with PBS (n=8) or 2% (n=2), 0.2% (n=5), or 0.02% (n=5) BZK. Baseline scores were similar (p=.25). In the sheep treated with 2% and 0.2% BZK, scores were significantly higher after treatment than scores in the PBS-treated sheep (p<0.0001). In the sheep treated with 0.02% BZK, there were no significant differences in the vagina when compared to PBS treated sheep (p=.96). (Error bars indicate 95% confidence intervals. Asterisks indicate statistical significance.)

Table 1

Comparison of sheep and human features of the cervix and vagina

	Sheep ^a	Human ^b
Cervical diameter	1-1.5cm	2.3-3.3 cm ^c
Vaginal length	9-13 cm	8-12 cm ^d
Vaginal epithelial layer thickness	86-114 µm	175-284 µm ^c
Vaginal epithelial cell layers	8-13	22-29 ^e
Vaginal pH	7.5-8.5	4.0-5.0 ^f

^aN = 6 (current study)

^bMeasurements obtained from published data

^cN = 16 (23)

^dN not available (25)

^eN = 74 (26)

^fN not available (27)

Colposcopy findings in the cervix and vagina at baseline (0 hr) and twenty-four hours (24hr) after a single treatment with 2%, 0.2%, or 0.02% BZK or PBS

Table 2

	2% BZK n=2		0.2% BZK n=5		0.02% BZK n=5		PBS n=8	
	No. (%) ^a		No. (%)		No. (%)		No. (%)	
	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Colposcopy findings								
Erythema	1 (50)	0 (0)	3 (60)	4 (80)	3 (60)	5 (100)	4 (50)	6 (75)
Edema/thickened tissue ^b	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Petechiae	1 (50)	0 (0)	2 (40)	4 (80)	1 (20)	5 (100)	1 (13)	3 (38)
Peeling ^c	0 (0)	2 (100)	0 (0)	3 (60)	0 (0)	0 (0)	0 (0)	0 (0)

^aNo. is the number of animals with the finding. Percent of animals with the finding is in parenthesis.

^bFindings with edema and thickened tissue involved global blanching and edema with loss of elasticity of the tissue. The normal vasculature seen in sheep was not seen with this finding so vascular disruption was indeterminate. In this study, this finding was always present with peeling.

^cFindings with peeling had intact vasculature and disrupted epithelium. No deep disruption was seen after a single dose treatment in any group.