Author's response to reviews

Title: Quantitative assessment of microbicide-induced injury in the ovine vaginal epithelium using confocal microendoscopy

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Author's response to reviews: see over
Dear Editors,

We thank you for consideration of our manuscript entitled “Quantitative assessment of microbicide-induced injury to the ovine vaginal epithelium using confocal microendoscopy.” We are pleased with the positive comments including the assessment that this article is of “outstanding merit and interest in its field.” The reviewer comments were quite helpful and we have made changes to the manuscript in response, we believe further improving the quality. We anticipate that this work will be of interest to your readership given the growing role of imaging in the field of infectious diseases and many other biological fields.

An item-per-item response is provided below. Changes were made in response to every requested item by the reviewer and we have streamlined the text to be more concise where possible. Again, we believe the manuscript is much improved.

Sincerely,

Gracie Vargas, Ph.D. – on behalf of the authors
Associate Professor, Department of Neuroscience & Cell Biology
Scientist, Center for Biomedical Engineering
Response to Reviewer Comments

Major Compulsory revisions:

1) The terms foreground and background were originally used as they are common image processing terms referring to pixels that are either part of the object of interest or in the near zero signal background. However, to reduce possible confusion, we altered the terms as requested. Because baseline generally is used in the context of a comparison (e.g. before/after) which was not what was meant, we chose to specifically state whether grayscale pixel values were above a threshold value (belonging to the object) or below the threshold value (subthreshold) in each image. We believe this removes confusion of terms such as foreground/background. These changes are found in the first full paragraph of page 8.

2) We have made attempts to be more concise throughout.

3) The change in wording to Page 1, results sentence 2 was made as suggested.

4) The reviewer was correct and we have modified this paragraph as suggested.

5) Changes regarding biopsy were made as suggested.

6) We have provided additional technical details to this section.

7) We have added to the text regarding potential clinical use of CFM. Current clinical use of CFM in GI and lung tissues is commented upon in the background (paragraph 4). As suggested, we have added comments regarding potential translation of CFM to clinical cervicovaginal imaging in the discussion section (last paragraph of Discussion section), noting that it is in fact dependent on the availability of non-toxic dyes. At the moment fluorescein is the most likely choice for clinical imaging, although a nuclear staining dye (acridine orange, primarily staining nuclei) is also being investigated in clinical trials of other tissues. No known live/dead cell fluorescent dyes are approved for clinical imaging, and future translation of CFM using live/dead cell dyes will be dependent on the development of non-toxic agents. Thus, we believe CFM as presented is best suited to preclinical studies in large animals, including in vivo longitudinal studies, but that clinical translation could occur most likely utilizing the widely accepted fluorescein given preclinical studies indicate it is useful in this field. Our group is currently investigating CFM based assessment of epithelial damage with fluorescein or acridine orange as contrast agents in the ovine model with hopes of future clinical translation. These points are discussed in the final paragraph of the Discussion.

8) An interesting point is raised by the reviewer. In fact in the study by Vincent et al., (STD 2009) changes in the ovine epithelium due to 0.02% BZK were not detected by colposcopy, whereas in the current study CFM did detect changes in the epithelium at this concentration, indicating CFM may allow one to detect damage not detected by the traditional method of colposcopy. This is now noted in the discussion on page 13, first paragraph. Whether CFM could detect damage by agents believed to by minimally cytotoxic & non-surfactant based agents remains to be explored (future evaluation of such has now been mentioned in conclusion section). We are encouraged by the results of this study showing damage at such a low concentration of BZK. Additionally, it is possible that other agents, while they may
not result in cell lysis, could still result in alterations such as epithelial structure, recruitment of infiltrates, or epithelial permeability. CFM could be coupled with any number of fluorescent agents to probe cellular/molecular changes given they can be targeted to appropriate markers of damage. For example, in the colorectum a recent study involved confocal endoscopy of mouse colon tissue labeled with a fluorescently labeled peptide targeting precancerous epithelium (Miller et al., PLoS One, 2011; 8(6)). Among considerations to be made are the target to be labeled, fluorophore, and the location/depth. CFM as shown in this study is restricted to the surface (~30 um total depth) which is now clarified in the text (as well as specifying this is a fixed-plane surface imaging approach on page 5, last sentence; However CFM with depth sectioning is also available, allowing imaging to depths of 80-250 um, however with slightly larger diameter endoscopic probes (5-7 mm) that could still be placed in the vaginal tract of large animals (and clinically if translated). The issue of the ability of CFM to image surface-restricted and subsurface fluorescent targets is now covered in the Discussion (page 14, last paragraph).

9) Identification of HEC has been added and referenced as suggested in the Methods (second paragraph).

10) Corrected

11) This change to the attributes of the CFM has been made as requested (in Abstract Conclusions)

12) The reviewer makes a good point that changes below the detection threshold of colposcopy may increase susceptibility to infection. We have emphasized this point as suggested (2nd paragraph of Background, 2nd-3rd sentences).

13) The word ‘gross’ has been taken out as requested.

14) The discussion on colposcopy disadvantages has been modified as suggested.

15) The use of ‘micromolar’ to describe concentration has been used as requested.

16) The technical description of the excitation/emission capabilities of the CFM have been clarified on page 7 to state specifically that one excitation wavelength is available with emission detected in the 505-700 nm spectral range (replaced term ‘single channel’)

17) The word Matlab has been added as requested.

18) This description has been clarified as suggested in the second sentence of Page 11.

19) We agree that it would be interesting to look into correlations at the site-to-site level and we are hoping to perform such correlations with a study providing more CFM sampling across a field, as opposed to separated 600 x 600 micron fields. By sampling several neighboring sites across the tract variations across epithelium could be better assessed. It is also worth mentioning that for longitudinal studies, an advantage of CFM is that many more samples can be obtained with the imaging probe than could be feasible with biopsy – an attribute particularly attractive to longitudinal studies (added sentence to 4th paragraph of discussion).