How to Use N-Acetylcysteine to Enhance Diagnosis of Bacterial Endometritis in Barren Mares

Karen A. Von Dollen, DVM, MS, DACT†; Karen Wolfsdorf, DVM, DACT; Samantha Levkulic, BS; Justine Elam, MT (ASCP); Carleigh E. Fedorka, PhD; and Kristina G. Lu, VMD, DACT*

Authors’ addresses: Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY, 40511 (Von Dollen, Wolfsdorf, Levkulic, Elam, Lu); Department of Veterinary Science, University of Kentucky, 1400 Nicholasville Road, Lexington, KY 40503 (Fedorka); e-mail: klu@hagyard.com.
*Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction

Endometritis is a leading cause of reproductive inefficiency in broodmares. Accurate diagnosis is critical in formulating an effective treatment plan. Multiple methods for obtaining endometrial samples for culture and cytology exist, with a range of associated sensitivity and specificity patterns.1–3 Endometrial sampling by low-volume lavage has been heralded for good-quality results4 and offers an advantage over swab/brush techniques through sampling of a greater surface area of the endometrium. This may be of particular advantage in mares with a history of subfertility. The presence of mucus within the equine uterus has been considered and explored as a factor influencing a mare’s reproductive soundness.5 N-acetylcysteine (NAC), a mucolytic that acts through disruption of disulfide bonds linking mucin, has been harnessed in equine reproductive practice as an antibiofilm agent6 and has been demonstrated to improve reproductive performance.7 Operating under the hypothesis that endometrial mucus may harbor bacteria and prevent their diagnosis through traditional sampling methods such as swab/brush or low-volume lavage, the impact of NAC infusion on uterine culture and cytology results was investigated.

2. Materials and Methods

Fifty-nine mares (58 Thoroughbreds and 1 Warmblood), barren from the 2020 breeding season, were evaluated between August and December 2020. Mares included were examined during the course of routine clinical practice in Lexington, KY.

Day 0

On day 0, 1-L of sterile lactated Ringer’s solution was instilled into the uterus and immediately collected back into the original bottle. Following this 1-L lavage, each mare was infused with 3.3% NAC (20 mL 20% N-acetylcysteine with 100 mL sterile 0.9% saline). A neat sample of lavage fluid (approximately 3 mL) was retained for turbidity analysis. The sample of neat lavage fluid was analyzed using a densimeter5 to obtain an objective measure of turbidity. A cuvette of water was used to zero the machine reading. Then, 1.8 mL of lavage fluid was added to a new cuvette and the
sample turbidity measured. The remaining uterine lavage fluid was submitted the same day for standard processing for culture and cytology. The 1-L bottle was allowed to rest undisturbed for one hour to encourage sediment formation at the bottom of the bottle. The sediment was aspirated using a pipette and divided into two 50-mL conical tubes for centrifugation. One centrifuged pellet was plated on Tryptic Soy Agar with 5% Sheep Blood, Columbia CNA Agar with 5% Sheep Blood, and MacConkey Agar, with the other prepared on a microscope slide and stained with Diff-Quik and Gram stain for cytologic evaluation. Bacterial growth was reported as no growth, scant (<10 colony forming units), light (growth on one quadrant of plate only), moderate (growth on two quadrants of plate), or heavy (growth on three quadrants of plate). While the cytology results were reported in more detail than white blood cell evaluation alone, cytology results were compared between patient samples based on the number of white blood cells present (none, rare: 0-1 per five high-power fields, few: 1-3 per high-power field, moderate: 6-10 per high-power field, many: >10 per high-power field). A negative cytology was defined as no or rare white blood cells present, with all others defined as a positive cytology result. To minimize operator variation, all cytology slides for this project were read by one of two trained laboratory technicians.

Day 1
The following day, each mare had the above lavage culture procedure repeated in an identical fashion (instillation of 1-L sterile lactated Ringer’s solution, collected immediately back into the original bottle). Laboratory sampling and processing procedures were performed as above.

Statistical Analysis
Statistical analyses were performed using SAS® 9.4 using varying models depending on the data. Quantitative/continuous data means were analyzed utilizing an independent-group T-test to make comparisons on means. Qualitative/categorical data were analyzed utilizing a chi-squared test for comparisons of proportions. Significance was set to P < 0.05.

3. Results
On day 0, 81% (48/59) of samples were cytologically negative, with 27% (16/59) cytologically negative the following day. This was not unexpected as any intraluminal manipulation of the uterus is likely to incite an inflammatory response. On day 0, 63% (37/59) of samples had no bacterial growth, 20% (12/59) had scant bacterial growth, 10% (6/59) had light bacterial growth, 7% (4/59) had moderate bacterial growth, and none had heavy bacterial growth. The following day, these bacterial growth profiles changed to 31% (18/59) no growth, 25% (15/59) scant growth, 15% (9/59) light growth, 24% (14/59) moderate growth, and 5% (3/59) heavy growth. Forty-eight mares had a negative cytology and no or scant bacterial growth on day 0. These 48 mares would reasonably have been designated “clean” by a practitioner based on these results. However, following NAC infusion, 17 of these mares (35%) displayed an inflammatory cytology and an increase in bacterial growth to light (5 mares), moderate (10 mares), or heavy (2 mares). Turbidity values were reported in million cells/mL and ranged from 0 to 135 for day-0 samples and 0 to 946 for day-1 samples. A threshold value of 50 million cells/mL was established as a cutoff for negative (below 50 million cells/mL) or positive (above 50 million cells/mL) densitometer results. Ninety-two percent (54/59) of day-0 samples were negative, and 75% (44/59) of day-1 samples were negative. Densitometer result was significantly associated with culture result, with 100% of those mares displaying an increase in densitometer reading from negative to positive also having an increase in bacterial culture grade (p < 0.01). However, if densitometer reading remained negative on day 0 and day 1, only 36% of those samples had an increase in bacterial culture grade (p < 0.05).

4. Discussion
The inclusion of NAC infusion prior to uterine lavage for culture and cytology has facilitated the diagnosis of a notable number of mares with bacterial endometritis that may otherwise have gone undetected by standard diagnostic methods. It is presumed that some mares produce a layer of mucus that acts to protect bacteria and prevent their detection, and only by disrupting this mucus layer are they able to be accurately diagnosed. Objective assessment of lavage fluid turbidity via densitometer was included in an attempt to relate the gross appearance of lavage efflux with laboratory findings. A very strong association between gross turbidity of a lavage and its likelihood of growing bacteria was found, making this an exciting potential adjunct diagnostic tool, especially in clinical settings that may have limited opportunities for performing cytology and must be selective in which samples are submitted for culture due to budgetary concerns. By no means is this diagnostic method proposed as a replacement for cytology and culture.

As this project was undertaken during clinical practice, there are inherent limitations to design and interpretation. First and foremost, no control groups were available to rigorously test the hypothesis. Control groups would have allowed for assessment of the influence of the low-volume lavage process itself on the following day’s results, as well as shed light on the potential role of any vaginal contamination during the lavage and infusion processes. Despite this drawback, the authors opine that this procedure has greatly enhanced the ability to diagnose (and subsequently treat) endometritis in mares.
Acknowledgments

The Authors are deeply grateful for the efforts of the Hagyard Laboratory Staff, whose skills are matched only by their kindness and willingness to collaborate on clinical research.

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnote
