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Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice

W.T. Riddle [a,*], M.M. LeBlanc [a], A.J. Stromberg [b]

[a] *Rood and Riddle Equine Hospital, Lexington, KY 40580, USA*
[b] *Department of Statistics, University of Kentucky, Lexington, KY 40506-0027, USA*

Abstract

Endometrial cytology and culture specimens ($n = 2123$) were collected concurrently with a guarded uterine culture instrument from 970 mares (738 barren, 1230 foaling and 155 maiden mares) during three breeding seasons (2001–2004). Results were compared to the 28-d pregnancy rate for the cycle from which the samples were taken. Cytological smears were evaluated for inflammation at ×100 and graded as: not inflammatory (0–2 neutrophils/field), moderate inflammation (2–5 neutrophils/field), severe inflammation (>5 neutrophils/field), or hypocellular (scant epithelial cells and no neutrophils). Uterine culture swabs were plated within 6 h, incubated for 72 h and results determined at 24, 48, and 72 h. Approximately, 20% ($n = 423$) cytology samples were positive for inflammation (>2 neutrophils), whereas approximately 11% ($n = 231$) of cultures had microorganisms recovered. A majority (64%) of the positive cultures (147/231) had inflammation on cytology smears. *Streptococcus equi* subsp. *zooepidemicus* was associated with more positive cytology results than coliforms ($P < 0.01$). Mares with positive cytology or culture had lower pregnancy rates than mares with normal findings ($P < 0.01$). Lowest pregnancy rates were recorded for mares with severe endometrial inflammation (21%, versus moderate inflammation 48%). Isolation of a microorganism from mares with endometrial inflammation was not associated with a further reduction in pregnancy rates. In barren, foaling and maiden mares, cytology was positive in 28, 17, and 5%, respectively, and culture was positive in 12.2, 11.1, and 3.2%. Foaling and maiden mares had higher pregnancy rates than barren mares (62, 69, and 44%, respectively, $P < 0.001$). In conclusion, a positive cytology was twice as common as a positive culture, and isolation of microorganisms was associated with reduced pregnancy rates, even in the apparent absence of inflammation.

Keywords: Mare; Uterus; Inflammation; Bacteria; Pregnancy rate

1. Introduction

The diagnosis of endometritis is critical in the veterinarian's attempts to treat infertility. Modalities that are available to diagnose endometritis include clinical examination, transrectal palpation and ultrasonography of the reproductive tract, vaginal speculum examination, uterine culture, cytology, and endometrial biopsy. Endometritis is most commonly associated with aerobic bacterial infection, but may also be caused by anaerobic bacteria, pneumovagina, urine pooling, exposure to semen, and intra-uterine infusion with an irritating substance.

Aerobic bacteriological culture of the uterus of mares is the most common method for diagnosing endometritis. However, isolation of bacteria does not necessarily prove the presence of endometritis nor does failure to isolate bacteria eliminate it [1,2]. Evaluation of cytological specimens in conjunction with endometrial culture has

* Corresponding author. Tel.: +1 859 233 0371; fax: +1 859 255 5367.
E-mail address: triddle@roodandriddle.com (W.T. Riddle).

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been promoted since the early 1980s as a technique that enhances the accuracy of endometrial culture in identifying mares with endometritis [3–13]. The correlation between the presence of neutrophils in cytological specimens and the isolation of uterine pathogens [3,4] was high when samples were collected with guarded uterine swabs. Although isolation of a microorganism from the uterus of a mare during estrus in the absence of cytological evidence of endometrial inflammation has been reported to falsely identify mares as having bacterial endometritis, no research studies could be found to support or refute this belief.

The Thoroughbred breeding farms in Central Kentucky require that all barren and maiden mares have a veterinary certificate stating that no uterine pathogens were isolated from the mare prior to the first mating. The majority of farms also require a uterine culture from all mares (barren, maiden, and foaling) before subsequent matings to ensure there are no uterine pathogens. However, it is often impossible to wait 48–72 h to ensure that no microorganisms are isolated from a uterine swab before mating. Some veterinarians collect an endometrial sample for cytological analysis concurrently with the culture swab because cytology results are available the same day as sampling. Endometrial cytology is also performed because some veterinarians consider uterine cytology to be more diagnostic for endometritis than bacterial culture.

A number of techniques for collection and interpretation of cytological samples have been reported [3–15]. Endometrial smears for cytological examination have been prepared with material collected with a gloved finger, a cotton swab or cap on a double-guarded culture swab, uterine biopsy, or from uterine lavage fluids. Endometrial inflammation has been quantified by counting the number of neutrophils per high power microscopic field, the number of neutrophils per slide, or determining the ratio between neutrophils and epithelial cells. Results have been categorized as either positive or negative for inflammation or graded as mild, moderate or severe inflammation. Our practice uses the endometrial cytology technique proposed by Asbury [6]; cytological specimens are prepared by smearing the endometrial fluid trapped in the cap of a Kalayjian culture instrument onto a glass slide. The slide is then either air-dried or fixed with a fixative. Smears are stained with a polychrome stain, and endometrial inflammation is graded by counting the number of neutrophils/field under oil immersion.

This retrospective field study was conducted to determine how endometrial cytological findings related to uterine swab culture results and the 28-d pregnancy rate. We hypothesized that pregnancy rates would be decreased in mares that had endometrial inflammation and in mares with endometrial inflammation and positive cultures.

2. Materials and methods

Endometrial cytology and culture swabs were collected from 970 Thoroughbred mares during the 2001–2004 breeding seasons. Samples were collected from two populations of mares. The first population consisted of mares that resided on farms visited daily by the first two authors. An endometrial specimen was collected for cytological analysis concurrently with a culture specimen from all mares on these farms (with the exception of maiden mares that did not necessarily have a cytology performed before their first mating). The second population consisted of mares that were repeat breeders, or were suspected to have endometrial inflammation or bacterial endometritis. Three ambulatory clinicians collected these samples. Pregnancy status 28 d after the breeding that followed collection of the culture and cytology was compared to uterine culture and cytology results. All pregnancy examinations were performed by ultrasonography. Twenty-eight day pregnancy rates for each cytology and culture pair were obtained from either reproductive records kept by the veterinarian or the billing records of the hospital. Results from subsequent pregnancy examinations were not considered because many pregnancies were lost during two of the breeding seasons (2001 and 2002) when an outbreak of Mare Reproductive Loss Syndrome affected the Kentucky horse breeding industry (MRLS losses occurred after 35 d of gestation). Fourteen and 21-d pregnancy rates were not used because early embryonic loss could have been associated with endometritis. Endometrial cytology and culture specimens were collected with Kalayjian swabs[1] when the mare was in estrus. The culture instrument was either passed through a sterile vaginal speculum into the uterus or manually inserted by a veterinarian wearing a sterile sleeve. Once the instrument was inside the uterus, the inner rod was advanced forward into the uterus to force open the cap on the end of the outer guard. The cotton swab on the end of the rod was rotated for 10–15 s inside the uterus and then withdrawn into the outer guard. The outer guard and its cap were then rotated for 10–15 s to collect cells within the cap. Once the instrument was withdrawn from the

[1] (Kalayjian culture instrument; Kalayjian Industries, Inc. Signal Hill, CA, USA).

vagina, the swab was immediately placed into a charcoal medium.[2] The cap was tapped on a microscope slide and a smear for cytological analysis was made using the cap. When secretions were thick, one author slid a second slide over the first in a manner similar to preparing a blood smear. The slide was than either fixed with a fixative spray[3] or air-dried.

Cytological specimens were stained with Diff Quick stain[4] and were evaluated microscopically under oil immersion (×100) by one of three medical technologists. A minimum of 10 fields was evaluated on each slide. Findings were categorized as follows: normal cytology—epithelial cells and 0–2 neutrophils/field; moderate inflammation—epithelial cells and 2–5 neutrophils/field; severe inflammation—epithelial cells and >5 neutrophils/× field; hypocellular specimen—scant epithelial cells on slide, no neutrophils, debris or bacteria present.

Endometrial cultures were plated on blood and Levine Eosin-Methylene Blue plates at the medical laboratory at Rood and Riddle Equine Hospital within 6 h of sample procurement. Plates were incubated at 37 °C and examined every 24 h for 3 d. Bacteria were identified with BBL crystals.[5] Antimicrobial sensitivities of all isolated organisms were determined by the Kirby Bauer method. Primary identification of yeast was performed by dark field microscopy. *Bacillus*, *Micrococcus* and alpha *Streptococcus* were listed as non-pathogenic organisms.

The attending veterinarian determined the breeding management. No mares bred on foal heat were used in this study. Mares were usually bred only once prior to ovulation. Ovulation was induced using either hCG[6] or deslorelin acetate.[7] Treatment after breeding varied among veterinarians. If cytological specimens were normal, some veterinarians infused antibiotics into the uterus at the time of detection of ovulation (usually the day after mating), whereas, others only administered oxytocin or did not treat. If cytological specimens exhibited inflammation or microorganisms were isolated after the mare was mated, mares were treated more aggressively after breeding. Aggressive treatment included uterine lavage, intra-uterine antibiotics and/or ecbolic drugs usually at 4–8 h after breeding, 24 h and occasionally 48 h after breeding. In some cases, if bacteria were isolated and there was no cytological evidence of inflammation, the uterus was only infused with the appropriate antibiotic (based on sensitivities) at the time of the ovulation check.

2.1. Statistical analysis

Each sampling period (paired culture and cytology) was considered a single event. Comparisons were made between pregnancy rates, culture results, cytology results, microorganisms recovered, and mare status. All probability values were computed using Pearson's Chi-Square statistic; $P < 0.05$ was considered significant.

3. Results

The study evaluated results of 2123 paired culture/cytology specimens and determined pregnancy rates for 1758 of the paired samples. The 365 samples not included in the pregnancy data set included 286 mares that were not bred on that estrus period and 79 mares with hypocellular findings on cytology. Uterine cytology and culture results were normal in 72% (1537/2044) of the mares (Table 1); these mares had a 60% pregnancy rate (Table 1). Mares with cytological evidence of inflammation and mares that had a microorganism isolated from the uterus had decreased pregnancy rates ($P < 0.01$), with lowest pregnancy rates in mares exhibiting severe inflammation (21%; $P < 0.01$). Isolation of a microorganism from mares with endometrial inflammation was not associated with a further reduction in pregnancy rates. Microorganisms were isolated from 19.7% of the mares with cytological evidence of moderate inflammation and from 49% of the mares with severe inflammation.

Whereas 423 of the cytological smears had evidence of inflammation (19.9%), a microorganism was isolated from only 231 culture swabs (10.8%; Table 2). Microorganisms were recovered from 90/738 (12.2%) barren mares, 136/1230 (11%) foaling mares and 5/155 (3.2%) maiden mares. The most common isolate was beta-hemolytic *Streptococcus* (78 samples, 34%), followed by *E. coli* (17%), *Pseudomonas* spp. (11.7%), and organisms considered to be non-pathogens (11.3%; Micrococcus, alpha *Streptococcus* and *Bacillus*). Two or more organisms were recovered from 9% of the culture swabs that had a microorganism recovered. The remaining 39 positive swabs (17%) yielded yeast, *Staphylococcus*, *Proteus*, *Enterobacter cloaca*, or *Klebsiella*.

[2] (Difco® Transport Medium Amies; Becton, Dickinson and Co.; Sparks, MD, USA).

[3] (Cytology Fixative Spray; US Biotech Corp., Webberville, KY, USA).

[4] (Diff Quik; Hemal Stain Co. Inc.; Danbury, CT, USA).

[5] (BBL crystals; Becton, Dickinson and Co.).

[6] (Chorulon; Intervet Inc.; Millosboro, DE, USA).

[7] (Ovuplant; Fort Dodge, Shawnee, KS, USA).

Table 1
Relationships between pregnancy rates, endometrial cytological findings and uterine culture swabs in Thoroughbred mares

	No. of mares	Pregnant (%)[a]	Not pregnant (%)	Not bred (%)[b]
Cytology 0–2 neutrophils[c]; no bacteria isolated	1537	865(60) a	571(40)	101(7)
Cytology 0–2 neutrophils; bacteria isolated	84	24(36) b	43(64)	17(20)
Cytology 2–5 neutrophils; no bacteria isolated	170	65(49) b	67(51)	38(22)
Cytology 2–5 neutrophils; bacteria isolated	43	11(44) b	14(56)	18(43)
Cytology >5 neutrophils; no bacteria isolated	107	14(23) c	46(77)	47(44)
Cytology >5 neutrophils; bacteria isolated	103	7(18) c	31(82)	65(63)
Total	2044[d]	986(56)	772(44)	286

Pregnancy rates with different letters (a–c) are different ($P < 0.01$).
[a] Percentage of pregnant mares was computed by dividing the number of pregnant mares by the number of mares bred.
[b] Percentage of mares not bred was calculated by dividing the number of mares not bred by the total number of mares.
[c] Number of neutrophils indentified at 1000× (minimum of 10 fields) were graded as: 0–2 neutrophils, normal cytology; 2–5 neutrophils/field, moderate inflammation; >5 neutrophils/field, severe inflammation.
[d] 79 of 2123 cytological samples were graded as hypocellular and are not included in the data set.

Isolation of a microorganism was not always associated with inflammation (Tables 1 and 2). Eighty-four of the cytological smears prepared concurrently with the 231 positive culture swabs had ≤2 neutrophils/field (36%; Table 1). Pregnancy rate in these mares was only 36%. Not all positive cultures were associated with positive cytologies (Table 2). Sixty-five percent of cultures from which beta-hemolytic *Streptococcus* was isolated and 80 and 82% of cultures with *Klebsiella* or *Staphylococcus* isolated, respectively, had cytological evidence of inflammation, whereas 50–58% of the *E. coli*, *Pseudomonas*, *Enterobacter cloaca,* yeast or organisms considered not to be pathogens were associated with inflammation. Isolation of ≥2 organisms was always associated with endometrial inflammation, with severe inflammation most commonly reported (76%).

Beta-hemolytic *Streptococcus*, *E. coli* and *Pseudomonas* represented 72% of the isolates recovered from foaling mares (Table 3). The types of microorganisms recovered from barren mares were more varied. Beta-hemolytic *Streptococcus* and organisms considered not to be pathogens were most commonly isolated (23 and 19%, respectively) followed by *E. coli* (13%), yeast (12%), ≥2 organisms (11%) and *Pseudomonas* (10%). Bacteria were isolated from only 5 of 155 endometrial samples collected from maiden mares, with beta-hemolytic *Streptococcus* being isolated from 4 of the 5 culture swabs and *Pseudomonas* from the remaining swab.

Table 2
Relationship between microorganisms isolated from uterine swabs and cytological findings in Thoroughbred mares

Bacteria	No. of inflammation[a]	Moderate inflammation[b]	Severe inflammation[c]	No. of cytologies with inflammation (%)[d]
Beta-hemolytic *Streptococcus*	27	9	42	51/78(65) a
E. coli	18	12	10	22/40(55) b
Pseudomonas	13	4	10	14/27(52) b
Non-pathogenic[e]	11	7	8	15/26 (58%) a,b
>2 organisms	0	5	16	21/21(100) c
Yeast	5	1	6	7/12(58) a,b
Staphylococcus	2	2	7	9/11(82) a,c
Proteus	4	0	3	3/7(43)[f]
Enterobacter cloaca	2	2	0	2/4(50)
Klebsiella	1	2	2	4/5 (80)

In the last column, rates without a common letter (a–c) differ ($P < 0.05$).
[a] 0–2 neutrophils/1000× field.
[b] 2–5 neutrophils/1000× field.
[c] >5 neutrophils/1000× field.
[d] Number of cytological specimens that had ≥2 neutrophils/1000× field in relation to the number of cultures from which bacteria were isolated.
[e] Isolation of *Bacillus*, *Micrococcus*, or alpha *Streptococcus*.
[f] Number of samples in population was too small to analyze statistically.

Table 3
Isolation of microorganisms from the uterus of barren, foaling and maiden Thoroughbred mares

Organism	Barren (%)[a]	Foaling (%)	Maiden (%)
Beta-hemolytic *Streptococcus*	21(23)	53(39)	4(80)
Non-pathogenic	17(19)	9(7)	
E. coli	12(13)	28(20.5)	
Yeast	11(12)	1(1)	
≥2 organisms	10(11)	11(8)	
Pseudomonas	9(10)	17(12.5)	1(20)
Proteus	3(3)	4(3)	
Staphylococcus	3(3)	8(6)	
Enterobacteria cloaca	2(2)	2(1)	
Klebsiella	2(2)	3(2)	
Total	90	136	5

[a] The number in parentheses is the percentage of samples from which the bacterial isolate was recovered, divided by the number of total samples within mare group (Barren, Foaling or Maiden).

Table 4
Day-28 pregnancy rates in Thoroughbred mares with microorganisms recovered from their uterus

Culture	No. of mares pregnant	No. of mares not pregnant
Beta-hemolytic *Streptococcus*	12(30)	28(70)
E. coli	10(40)	15(60)
Pseudomonas	8(44)	10(56)
Non-pathogenic	5(25)	15(75)
>2 organisms	1(25)	3(75)
Yeast	0	11(100)
Staphylococcus	3(50)	3(50)
Klebsiella	2(67)	1(33)
Proteus	1(50)	1(50)
Enterobacteria cloaca	0	2(100)
Total	42(32)	89(68)

One-hundred-thirty-one mares were bred on the cycle that a microorganism was isolated (Table 4). Mean 28-d pregnancy rate in these mares was 32% (42/131). No mares that harbored yeast in their uterus were pregnant at 28 d (0 of 11 mares), whereas 50% of the mares (3 of 6) with a *Staphylococcus* isolated were pregnant. Twenty-eight day pregnancy rate was 25% when ≥2 organisms or an organism considered not to be a pathogen was isolated from the uterus.

Foaling and maiden mares had higher pregnancy rates than barren mares ($P < 0.001$). The 28-d pregnancy rates for barren, foaling and maiden mares were 44% (263/594), 62% (683/1094) and 69% (102/148), respectively. Ninety-five percent of maiden mares were bred on the cycle that the paired cytology and culture were collected, as compared to 80.5% of the barren and 89% of the foaling mares. The relationship between cytological findings and mare status is shown in Table 5. Maiden mares had the highest number of cytological samples graded as hypocellular ($n = 44/79$, 55.7%), had the smallest number of samples with evidence of inflammation ($n = 8$) and had the fewest positive endometrial cultures ($n = 5$). Of the 423 cytological smears with evidence of inflammation, 210 (49.7%) were recovered from barren mares, 205 (48.4%) from foaling mares, and eight (1.9%) from maiden mares.

4. Discussion

Both endometrial cytology and culture were diagnostic for identifying mares with endometritis, as pregnancy rates were decreased when either test was positive. However, endometrial cytology identified twice as many mares with endometritis as did endometrial culture. This finding was similar to that of Wingfield Digby [4], who reported that 91% of mares with clinical evidence of persistent endometritis had cytological evidence of endometritis, but only 45% had relevant bacteriological findings. Endometrial culture

Table 5
Relationship between cytological findings and mare status

	No. of barren mares (%)[a]	No. of foaling mares (%)	No. of maiden mares (%)	Total (%)[b]
Cytology 0–2 neutrophils[c]	521(70.6) a,b	997(81) a	103(66.5) b	1621(76.4)
Cytology 2–5 neutrophils	112(15) a	95(8.1) a	6(3.8) b	213(10)
Cytology >5 neutrophils	98(13) a	110(9) a	2(1.3) b	210(9.9)
Hypocellular	7(0.9) a	28(2.3) a	44(28.4) b	79(3.7)
Total	738	1230	155	2123

Within a row, rates without a common letter (a and b) differ ($P < 0.05$).
[a] Percentage represents the number of mares within status (Barren, Foaling, or Maiden).
[b] Percentage of mares in the total column are the number of mares in the row divided by the total number of mares.
[c] Number of neutrophils indentified at 1000× (minimum of 10 fields) were graded as: 0–2 neutrophils, normal cytology; 2–5 neutrophils/field, moderate inflammation; >5 neutrophils/field, severe inflammation.

swabs may not identify all mares with bacterial endometritis. Nielsen [15] recently showed that the sensitivity of endometrial swabs in isolating aerobic microorganism from the uterus was only 0.44 when results were compared to cultures taken from endometrial biopsy. The discrepancy between endometrial swab findings, endometrial cytology or culture of an endometrial biopsy may be associated with inadequate sampling of the endometrium [15], technique, non-infectious causes of endometritis (e.g. pneumovagina or vestibule-vaginal reflux), presence of anaerobic bacteria, or bacterial secretions. When a cytological sample is collected with a Kalayjian swab, only a small area within the dorsal uterine body is sampled. As intrauterine fluid most commonly collects in the uterine horns, this area may not be the most appropriate site for collection of uterine cultures in older barren mares or in foaling mares. The technique used in this study produced adequate endometrial cytological samples because only 79 of 2123 cytological smears (3.6%) were hypocellular, with the majority collected from maiden mares. Maiden mares are more likely to have a cervix that is not completely relaxed, resulting in the possibility of a cervical, rather than a uterine sample being taken when the sample is procured through a vaginal speculum. In addition, uterine secretions in maiden mares may be low, especially if the sample was collected in early estrus. Samples collected early in estrus may not contain inflammatory cells as neutrophil migration into the uterine lumen during the period of waning progesterone dominance is lower than during maximal estrogen dominance.

The technique of making the cytology smear varied slightly among veterinarians, with one veterinarian thinning uterine secretions across the glass slide in a manner similar to making a blood smear, whereas the others tapped the cap on the slide to make the smear. Perhaps some cytology smears had neutrophils present, but the smears were too thick for the cytologist to identify cells accurately. We did not evaluate if the number of negative cytology/positive culture pairs differed among investigators, nor did we grade cytological debris. We agree with Card [14] who noted that cytological smears taken from some subfertile mares contain large amounts of debris and may or may not contain inflammatory cells [14]. The debris may represent degenerate neutrophils, sloughed epithelial cells, and inflammatory residue. Smears with large quantities of debris were noted by our cytologists; however, we recorded them as not inflammatory if there were <2 neutrophils/field. Anaerobic bacteria may have resided in the uterine lumen and were not identified by our culture technique. Ricketts isolated anaerobes from 84 guarded uterine swabs taken from 263 Thoroughbred mares; however, only 12% (10/84) were associated with cytological evidence of endometritis [15].

The type of exudate produced by microorganisms may influence the amount of inflammatory cells on a cytological specimen. Dimock and Edwards [17] noted almost 100 years ago that mares infected with *Streptococcus equi* subsp. *zooepidemicus* could be differentiated from mares with *Klebsiella* infections by examining the exudate present in the vagina. Mares infected with *Streptococcus* had either a large quantity of a very thin, slightly cloudy muco-purulent material in the vagina or a thin, clear, slightly frothy exudate coming from the cervix. In contrast, mares infected with *Klebsiella* had a thick, exceedingly viscid and tenacious exudate. If a pathogen produces a thick mucoid exudate, it would be difficult to identify inflammatory cells on cytological smear. In this study, the percentage of cytological specimens graded as inflammatory varied among the microorganisms recovered. Isolation of beta-hemolytic *Streptococcus*, *Staphylococcus,* or ≥ 2 organisms from the uterus were most commonly associated with cytological smears graded as severe. In contrast, isolation of *Pseudomonas*, coliforms (*E. coli* and *E. cloaca*) and *Proteus* were more likely not to have cytological evidence of inflammation.

A number of parameters in this retrospective study that may influence pregnancy rates were not controlled and therefore need to be considered. The percentage of positive cultures and cytologies in this study do not represent a normal distribution pattern for Thoroughbred mares because: (1) the data set did not include maiden mares bred on their first estrus, because cytologies are not routinely performed before a maiden's first breeding; (2) three of the five veterinarians only performed cultures and cytologies on ''problem mares''. The inclusion of barren mares bred on a positive cytology or culture and exclusion of first trip maiden mares skewed the pregnancy data from expected norms. Furthermore, the effects of stallion or individual mare management may have influenced pregnancy rates; however, the large number of mares included in the population and the number of mares each stallion bred in a season reduces their effects. Stallions in this study were mated to ≥ 70 mares each season and all had seasonal pregnancy rates of $\geq 80\%$. Medical management differed somewhat among veterinarians, however, it was routine practice among the five veterinarians to irrigate the uterus of any mare with a positive cytology or positive culture. Whereas all veterinarians would instill antibiotics into the uterus of

mares with positive cytologies, the type of antibiotic used may have differed.

Twenty-eight day pregnancy rates and not 14-d pregnancy rates were measured because pregnancy loss between 14 and 28 d is commonly associated with endometritis. We would have preferred measuring 60-d pregnancy rates to have a more complete picture of early embryonic death, but the presence of Mare Reproductive Loss Syndrome prevented us from doing so (MRLS is associated with pregnancy loss after 38 d of gestation).

The 28-d pregnancy rate decreased significantly among the three cytology categories (no inflammation, moderate and severe). Mares with 2–5 neutrophils/field were twice as likely to be pregnant at 28 d as mares with >5 neutrophils/field (46.5% versus 20.5% pregnancy rate at 28 d, respectively; $P < 0.01$). Mares with 0–2 neutrophils/field on cytological smears had pregnancy rates that were 1.3 and 3 times higher than those of mares with cytological smears exhibiting moderate or severe inflammation ($P < 0.01$; Table 1). Veterinarians must decide whether it is best to breed a mare with cytological evidence of inflammation on a particular cycle, or if it is best to identify the source of inflammation, treat the problem, and then breed on a subsequent cycle. Others have shown increased pregnancy rates in Standardbred mares treated during the estrus that endometrial inflammation was identified and later bred on a subsequent cycle [11]. In this study, mares with cytological evidence of moderate or severe inflammation before mating were treated aggressively after breeding with uterine lavage, ecbolics and intra-uterine or systemic antibiotics. Although pregnancy rates of mares with moderate inflammation on cytological smears may be considered acceptable (46.5%), they were quite low in mares with severe endometrial inflammation (21%), indicating that it may be best to treat a mare with severe inflammation on the affected cycle and breed on a subsequent cycle.

Pregnancy rate was only 36% in the 67 mares that had no cytological evidence of inflammation but had a uterine microorganism recovered (Table 1). It is unlikely that the microorganisms isolated from these mares were contaminants, because pregnancy rates were significantly lower than those of mares with no endometrial inflammation and negative culture results (36% versus 60%, respectively; Table 1). The attending veterinarian would have treated all 67 mares as soon as it became known that a pathogen was recovered. Contamination of swabs was also unlikely because microorganisms were only recovered from 5 of 155 maiden mares and the cytological smears prepared concurrently from these five mares all exhibited inflammation. In maiden mares, bacterial infection may have been a consequence of a previous breeding, because the majority of these mares would have been previously mated at least once.

The types of bacterial pathogens isolated from the endometrium of mares in this study were similar to those reported in earlier studies [4,16–21]. We had a larger percentage of foaling mares with *Pseudomonas* than previously reported by others. Not surprisingly, a more varied group of microorganisms were recovered from barren mares than from foaling mares. Of concern was the low pregnancy rate in mares (25%) that had what our laboratory considered to be non-pathogens (*Micrococcus*, *Bacillus* and alpha-hemolytic *Streptococcus*) recovered from culture swab. *Micrococcus* and *Bacillus* are considered skin commensals and were more commonly isolated from barren mares (14 barren, 8 foaling, and 0 maiden mares). Lack of an aggressive treatment strategy after breeding in these mares may have contributed to the low pregnancy rates. These mares would have received intra-uterine antibiotics at ovulation and in some cases uterine lavage and ecbolics 4–8 h after breeding. It is also possible that *Bacillus* and *Micrococcus* were not the cause of the lower pregnancy rate, but were associated with conditions that would decrease fertility. Barren and older foaling mares may have breaches in the physical barriers that protect against contamination, the vulvar seal, the vestibulo-vaginal fold and the cervix, making them more susceptible to infection by skin commensals and organisms that reside in the clitoris and vestibule.

In conclusion, pregnancy rates were affected by endometrial cytology and culture results. Twice as many mares were identified as having endometritis by endometrial cytology than by culture swab, with the degree of inflammation more important in diagnosing infertility than the mere presence or absence of inflammation (defined as >2 neutrophils). Isolation of microorganisms was always associated with decreased pregnancy rates, regardless of cytological findings. The uterine inflammatory response, as identified in cytological smears, appeared to differ among microorganisms, with beta-hemolytic *Streptococcus* being associated with a higher number of positive cytologies than coliforms. Finally, clinicians should always interpret their laboratory results with caution, unless clinical data indicate the presence of endometritis.

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