Update on antimicrobial therapy in mare reproduction

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Bacterial uterine infections are associated with significant time and monetary loss in the equine breeding industry. The incidence of identified bacterial uterine infection is estimated at 25 to 60% of barren mares.[1] Treatment and resolution of mare urogenital infection involves a holistic approach which must include consideration of the systemic health of the mare, correction of urogenital anatomic defects, enhancement of uterine clearance in non-pregnant mares, identification of specific infections, and appropriate antimicrobial selection and administration.

The following is a brief review of antimicrobial properties and classes, and discussion regarding antimicrobial selection for use in specific mare reproductive disease states. Excellent additional resources are available.[2] In this age of increasing bacterial and fungal antimicrobial resistance and heightened awareness of antimicrobial use in animals, judicious and wise employment of antimicrobials should be emphasized.

Keywords: Antibiotics, antifungals, chelators, endometritis, placentitis

General antimicrobial properties

Bactericidal antimicrobials

a) Time dependent (there is no benefit in increasing the drug concentration above the minimum inhibitory concentration (MIC); instead the time the drug concentration is above MIC is most important); examples are beta-lactams and trimethoprim-sulfonamide

b) Concentration dependent (rate of bacterial killing increases as the peak concentration increases above the pathogen's MIC rather than the duration of the drug's concentration); examples are aminoglycosides, fluoroquinolones, metronidazole, and peptides

Bacteriostatic antimicrobials
Concentration dependent; examples are macrolides, tetracyclines, and chloramphenicol

Antimicrobial classes

Beta-lactam: penicillin

Commonly used formulations are procaine penicillin, aqueous penicillin salts, ampicillin, ticarcillin; those less commonly used in equine reproduction include carbapenems (imipenem, meropenem)

Mechanism of action. Beta-lactams are time-dependent antimicrobials that interfere with cell wall formation by inhibiting the penicillin-binding proteins that catalyze polymer cross-linking necessary for cell wall formation. Beta-lactams are bactericidal for replicating cells, thus clinical benefit is reduced when used concurrently with a bacteriostatic drug. Penicillins are generally most effective against gram-positive organisms, however they vary in efficacy against gram-negative organisms. Differences in organism susceptibility are attributable to differences in penicillin-binding protein receptor sites, the amount of peptidoglycan present in the cell wall, drug penetration of the outer cell membrane of gram-negative bacteria, and beta-lactamase-induced resistance.

Distribution. Penicillins are well distributed throughout the body, reaching MIC in most organs including the reproductive tract. They are ionized, poorly lipid soluble, and tend to cross biological membranes poorly. They are excreted almost entirely by the kidney and thus can be useful to treat urinary tract infections caused by sensitive organisms.

Synergism. Beta lactams are synergistic with aminoglycosides

Evaluation of endometrial ampicillin concentration 24 hours after a three-gram intrauterine infusion of ampicillin revealed drug concentrations above MIC for investigated isolates 24 hours after infusion.[3] Intrauterine infusion of 6.2-g ticarcillin/clavulanic acid resulted in endometrial tissue concentrations of 150-424 micrograms/g 60 minutes after infusion. Clearance is rapid, potentially necessitating frequent administration to maintain concentrations.[4] Systemically administered potassium penicillin achieved MIC for *Streptococcus equi* subspecies *zooepidemicus* (hereafter *S. zooepidemicus*) in allantoic fluid.[5] Penicillins are also used for treatment of leptospirosis.

Addition of Timentin® (ticarcillin plus clavulanic acid) to Inra96® semen extender to a final concentration of 0.5 to 1.5 mg/mL can limit the growth of *Taylorella equigenitalis.*[6] Addition of Timentin® to semen extender (1 mg/mL) did not impair sperm quality but did not provide added protection against bacteria commonly found in equine semen.[7]

Beta-lactams: cephalosporins

Commonly used formulations are ceftiofur sodium, ceftiofur crystalline free acid, ceftiofur hydrochloride

Mechanism of action: Cephalosporins are also beta-lactam antimicrobials, however the molecular structure of the cephalosporin group of antimicrobials make them inherently more resistant to beta-lactamases. Cephalosporins are grouped in their order of development. Ceftiofur is a third generation cephalosporin in common usage in equine medicine. Ceftiofur is rapidly metabolized by plasma esterases to the active metabolite, desfuroylceftiofur.

Distribution. Widespread distribution into tissues in extracellular fluid is achieved however passage across membranes and physiological barriers is poor. Excretion is largely through the urinary tract.

Uses in reproduction. In mares administered ceftiofur sodium (2 mg/kg IM BID), ceftiofur sodium was not detected in endometrial tissue after the fifth dose when steady state concentrations were achieved.[8] In mares administered ceftiofur hydrochloride (2.2 mg/kg IM), a ceftiofur derivative was detected in endometrial tissue 24 hours after administration at concentrations above reported MIC for *S. zooepidemicus* and *Escherichia coli.*[9] In mares administered ceftiofur crystalline free acid (6.6 mg/kg IM), endometrial drug concentrations remained above MIC for *S. zooepidemicus* for 96 hours.[10] In a study evaluating efficacy of ceftiofur crystalline free acid in equine placentitis, the drug was not found in effective concentrations in the placenta, fetal tissue, amnionic fluid or fetal serum and did not improve foal survival rates.[11] Intrauterine ceftiofur sodium (1 g in 100 mL saline) has been found to be safe and effective.[12]

Beta-lactamase inhibitors

Clavulanic acid, a broad-spectrum beta-lactamase inhibitor, is used in combination with other antimicrobials such as ticarcillin. Certain bacteria can resist the action of beta-lactam antimicrobials by producing beta-lactamases, enzymes that degrade beta-lactam antimicrobials by opening the beta-lactam ring. Genes encoding beta-lactamase are transmitted through bacterial populations with plasmids and transposons as an important mechanism of spreading resistance. Beta-lactamase inhibitors bind irreversibly to beta-lactamases allowing the accompanying beta-lactam antibiotic to bind to the penicillin binding protein.

Peptide antibiotics

A commonly used formulationis polymyxin B

Mechanism of action. Polymyxins are peptide antibiotic products of *Bacillus polymyxa*. Polymyxin B is toxic at systemic antimicrobial doses with nephrotoxic, neurotoxic, and neuromuscular blocking effects, thus it is used topically for its antimicrobial properties and used systemically at sub-antimicrobial doses for binding and inactivating endotoxin (lipopolysaccharide). When used as an antimicrobial, polymyxin B is bactericidal and concentration-dependent. This cationic peptide disrupts the outer membrane of gram-negative bacteria by binding lipopolysaccharide (LPS), and increasing cell permeability. Gram-positive bacteria are resistant.

Distribution. Polymyxin diffuses poorly through biologic membranes and attains low concentrations in transcellular fluids.

Uses in reproduction. Polymyxin B can be infused into the uterus for gram-negative bacterial infections and used systemically for endotoxemia as may be seen in post-foaling complications.

Macrolides

Macrolides (erythromycin, azithromycin, clarithromycin) are predominantly bacteriostatic antimicrobials that inhibit protein synthesis by reversibly binding to the 50S ribosomal subunit, and thus are specific for prokaryotic cells. In adult horses, macrolide use can be associated with severe diarrhea. Macrolides are infrequently used in mare reproduction and will certainly be highlighted in discussion on antimicrobial use in foals and weanlings.

Aminoglycosides

Commonly used formulations are entamicin and amikacin; others include kanamycin, neomycin, streptomycin, dihydrostreptomycin, tobramycin.

Mechanism of action. Aminoglycosides are bactericidal, concentration dependent antimicrobials used primarily to treat aerobic gram-negative bacteria and staphylococci. Their bactericidal action is influenced by pH, being most active in alkaline pH. Aminoglycosides must penetrate bacterial cell walls, with penetration enhanced when in combination with drugs that interfere with the cell wall (e.g., penicillins). Once within the bacteria, they bind to the 30s ribosomal subunit (prokaryotic) and inhibit bacterial protein synthesis. The most clinically important resistance is due to enzymes from plasmids that are transferrable between bacteria. Elimination is by renal excretion (glomerular filtration) and nephrotoxicity is an important side effect. Risk factors for aminoglycoside toxicity include prolonged therapy (>7-10 days), multiple doses per day, acidosis, electrolyte disturbances, dehydration, concurrent nephrotoxic drug therapy, age, pre-existing renal disease, and elevated plasma trough concentrations. Ototoxicity may also occur. Amikacin has the broadest spectrum of the aminoglycosides, and is the least nephrotoxic. Concurrent administration of gentamicin with phenylbutazone was found to decrease elimination half-life of gentamicin by 23% and decrease the volume of distribution by 26% while pharmacokinetics of phenylbutazone were not affected. Aminoglycosides are pharmaceutically incompatible with many beta-lactams and should not be mixed in same syringe.[2]

Distribution. Aminoglycosides are large polycationic molecules that are poorly lipid soluble, with limited ability to enter cells and penetrate cellular barriers unaided. Purulent or necrotic debris bind and inactivate aminoglycosides.

Synergism. Aminoglycosides are synergistic with beta-lactams and trimethoprim-sulfonamide.

Antagonism. Antagonism may occur with chloramphenicol, tetracycline, erythromycin; should not be mixed with penicillins *in vitro.*[2]

Uses in reproduction. Intravenous gentamicin (6.6 mg/kg IV SID) yielded concentrations in allantoic fluid above MIC for potential gram-negative pathogens such as *E. coli*.[5] Amikacin is labeled for intrauterine use at a dose 2 g in 200 ml saline q 24 hrs for three consecutive days for endometritis, metritis, and pyometra in mares. Gentamicin (2 g in 80 mL saline) intrauterine infusion has been associated with shorter duration histologic inflammation though more cellular changes were seen with scanning electron microscopy compared to saline controls.[13,14] It is generally recommended that gentamicin be buffered or well diluted in saline to minimize irritation.

Tetracyclines

Commonly used formulations are oxytetracycline and doxycycline

Mechanism of action. Tetracyclines are broad spectrum, bacteristatic antimicrobials that diffuse through the outer cell membrane to reversibly bind to the 30s ribosomal subunit to inhibit protein synthesis. They are strong chelating agents with the ability to chelate divalent and trivalent ions such as calcium, with potential to cause tooth discoloration. There is widespread acquired resistance particularly among gram-negative bacteria and variable susceptibility in many *Staphylococcus sp*., *Streptococcus sp*., *Enterococcus sp*., *E. coli*, and *Klebsiella sp*. Resistance is rare in obligate intracellular pathogens such as *Ehrlichia sp*. and *Anaplasma sp*. Doxycycline (semi-synthetic) is more lipid soluble than oxytetracycline. Tetracyclines are relatively safe, with reported risks of enterocolitis, occasional fatal anaphylaxis, and collapse if intravenous administration is not slow. Renal tubular damage may occur if outdated product used.

Distribution. Tetracyclines are widely distributed.

Synergism. Tetracyclines are synergistic with polymyxins by enhancing bacterial uptake

Uses in reproduction. After intragastric administration of doxycycline (five doses, 10 mg/kg BID), the endometrial concentration was approximately 1.3 micrograms/mL, above the MIC for the evaluated strains of *S. zooepidemicus* and *Staphylococcus aureus.*[15] Tetracyclines can be used systemically for treatment of leptospirosis. In a study evaluating the efficacy and safety of intrauterine oxytetracycline, six grams of oxytetracycline was infused daily for three days. Endometrial oxytetracycline concentration remained above MIC for eight hours after infusion for *S. zooepidemicus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *E. coli*, with observation of transient endometrial inflammation, and thus the recommendation for further evaluation prior to routine intrauterine use.[16]

Chloramphenicol

Mechanism of action. Chloramphenicol is a bacteriostatic, lipid soluble, neutral, broad spectrum antimicrobial. It irreversibly binds to the 50S subunit of bacterial ribosomes and also inhibits mitochondrial protein synthesis is mammalian bone marrow cells in a dose-dependent manner. Resistance occurs via inactivation by chloramphenicol acetyltransferases (CATs) shared via plasmids, transposons, and integrons. Elimination occurs through the liver. Chloramphenicol is associated with idiosyncratic aplastic anemia in humans and is restricted in some countries because of bacterial resistance and risk of aplastic anemia.

Distribution. Chloramphenicol is widely distributed.

Uses in reproduction. Anecdotally used for intrauterine infusion when proteinaceous debris is present (W. Zent, personal comminication). Systemic use is based on clinical necessity.

Sulfonamides

Commonly used formulations are sulfadiazine, sulfasalazine, and sulfamethoxazole

Mechanism of action. Sulfonamides are bacteriostatic when used alone and bactericidal when combined with trimethoprim. They interfere with biosynthesis of folic acid in bacterial cells by competitively preventing para-aminobenzoic acid (PABA) incorporation into folic acid molecule by competing with PABA for dihydropteroate synthetase.

Bacteriostatic action depends on the requirement for folic acid; susceptible microorganisms must synthesize folic acid while mammalian cells use preformed folic acid. Tissue exudate or necrotic tissue must be removed for penetration.

Bacterial resistance is extensive. Elimination occurs through a combination of renal excretion and metabolic transformation. Adverse side effects, though uncommon, may include urinary tract disturbances, hematopoietic disorders, dermatologic disorders, and keratoconjunctivitis sicca in dogs.

Distribution. Sulfonamides are widely distributed.

Uses in reproduction. Systemic administration of trimethoprim sulfamethoxazole (30 mg/kg PO BID) in combination with pentoxifylline and altrenogest have been well evaluated in placentitis models (see below).

Diaminopyrimidines

A commonly used formulation is trimethoprim in conjunction with sulfonamides.

Mechanism of action. Trimethoprim interferes with folic acid production by inhibition of dihydrofolate reductase with greater affinity for bacterial versus mammalian enzymes, preventing synthesis of purines and thus DNA. They are bacteriostatic when used alone. Efficacy is decreased with plasmid associated resistance, and antagonism by tissue debris.

Fluoroquinolones

Commonly used formulations are enrofloxacin and ciprofloxacin.

Mechanism of action. Fluoroquinolones are concentration dependent, bactericidal antimicrobials that bind DNA-gyrase-DNA complexes to impair negative supercoiling and target DNA topoisomerase, disrupting replication. They have activity against a wide range of gram-negative bacteria, with ciprofloxacin being most potent against *Pseudomonas sp.* The drug is concentrated within phagocytic cells and largely excreted in urine. Protein synthesis inhibitors such as chloramphenicol and RNA synthesis inhibitors such as rifampin may reduce efficacy.

Resistance is not thought to be plasmid associated. Enrofloxacin inhibits cell proliferation, induces morphological changes and alters equine tendon structure, more pronounced in juvenile tendon cells. Arthropathies have been documented in two-week old foals, though not in adults. Fluoroquinolones are not recommended in pregnant animals, though they have been used in a pregnant mare with no known detrimental effect on the foal.[2] Transient neurologic signs have been seen with rapid administration.

Distribution. Fluoroquinolones are widely distributed.

Synergism. Fluoroquinolones are synergistic with beta-lactams, aminoglycosides, and vancomycin.

Antagonism. *In vitro* antagonism is seen between ciprofloxacin and chloramphenicol and ciprofloxacin and rifampin.

Uses in reproduction. Systemic enrofloxacin (5 mg/kg IV) achieved sufficient endometrial tissue concentrations to be used to treat endometritis caused by susceptible bacteria.[17] Enrofloxacin (5 mg/kg IV) has also been proposed as a therapeutic strategy for the prevention of endometritis is susceptible mares with a recommended dose (5 mg/kg IV) pre-breeding followed by two further doses 36 to 48 hours post-breeding.[18] Systemic ciprofloxacin (2.5 g PO SID) and probenecid (1 g PO SID) has been reported to effective against *Pseudomonas* infection.[19]

Intrauterine infusion of enrofloxacin has been evaluated with one author reporting enrofloxacin concentrations remaining above MIC for susceptible bacteria 24 hours after infusion of 2.5 mg/kg enrofloxacin.[20] A more recent study found endometrial biopsy grades worsening from Kenney Doig grade I to grade III over a 60 day period following intrauterine infusion of 2.5 mg/kg enrofloxacin daily for three days and concluded that enrofloxacin is not suitable for conventional intrauterine infusion treatment in mares.[21]

Nitroimidazole

A commonly used formulation is metronidazole.

Mechanism of action. Metronidazole undergoes reduction of the nitro group to yield unstable intermediates some of which interact with bacterial or protozoal DNA; the reduction occurs under anaerobic conditions. Resistance is rare among usually susceptible bacteria. Anorexia can be seen with oral use. Occasional neurological signs have been reported in other species with recovery facilitated by diazepam.

Distribution. Metronidazole is rapidly and well absorbed and lipophilic. It is metabolized in the liver and eliminated in the urine and manure. Hepatic metabolism may be decreased when concurrent with cimetidine, thus delaying elimination.

Uses in reproduction. Uncommonly used unless an anaerobic infection in suspected. After systemic administration of metronidazole (15 mg/kg loading followed by 7.5 mg/kg q6h via nasogastric tube), the mean endometrial concentration of metronidazole was approximately 0.9 micrograms/mL.[22]

Rifamycins

A commonly used formulation is rifampin.

Mechanism of action. Rifampin is bactericidal, broad spectrum, and active against both extracellular and intracellular pathogens. It inhibits DNA-dependent RNA polymerase. Because of rapid development of resistance, rifampin is usually administered in conjunction with other antimicrobials.

Distribution. Rifampin is very lipophilic and penetrates most tissues including abscesses, bone, milk and the central nervous system. Rifampin crosses the placenta and is teratogenic in rodents. Rifampin is eliminated via the liver.

Uses in reproduction. Uncommonly used in reproduction but can be useful in reproductive tract associated abscesses (e.g. abscessed broad ligament hematomas or retroperitoneal abscesses).

Antifungals

The main sites of action of antifungal drugs are the cytoplasmic membrane (polyenes, azoles), the cell wall, and DNA or protein synthesis.

Polyenes

Commonly used formulations are amphotericin B, natamycin, and nystatin.

Mechanism of action. Polyenes bind ergosterol, the principal sterol of the fungal cell membrane, causing leakage of cell contents. They also bind cholesterol in mammalian cell membranes (less avidly) which makes them the most toxic of clinically used antifungals. Polyenes are associated with renal toxicity. Amphotericin B is the mainstay for systemic fungicidal treatment of filamentous fungal infection. It is poorly soluble in water, unstable at 37°C, with maximal antifungal effects at pH 6-7.5, and decreased effects at low pH. Amphotericin B is not well absorbed orally and is thus intravenously administered. Nystatin has greater nephrotoxicity and is thus used topically (e.g. intrauterine).

Uses in reproduction. Polyenes are a reasonable first choice for treatment of fungal endometritis caused by yeast. Molds are most susceptible to polyenes and less susceptible to imidazoles and triazoles.[23]

Azoles

Commonly used formulations are imidazoles (clotrimazole, ketoconazole, and miconazole) and triazoles (itraconazole, fluconazole, and voriconazole).

Mechanism of action. Azoles inhibit cytochrome p450 dependent ergosterol synthesis leading to disruption of fungal membranes. Azoles are fungistatic, but fungicidal at high concentrations. Ketoconazole is poorly water soluble, lipophilic, requires acid pH, requires nasogastric tube administration (if administered in conjunction with HCl as recommended, the suspension can be irritating to the oral cavity and throat). Oral administration at 30 mg/kg does not result in detectable serum concentrations.[2] Itraconazole is poorly water soluble, lipophilic, and requires acid pH for absorption. Fluconazole is water soluble, oral absorption is unaffected by acid and it is well absorbed after oral administration, it distributes widely to tissues with the half-life in horses ~40 hours, and with 100% oral bioavailability. It can be administered orally or intravenously, though resistance is more frequent.

Voriconazole is a second generation triazole, with a wide spectrum, can be administered orally or intravenously, is metabolized by liver, with excellent tissue penetration, though with limited experience in horses.

Topical azoles

Commonly used formulations are clotrimazole and miconazole.

Uses in reproduction. Ketoconazole and clotrimazole are reasonable empiric treatment choices for uterine yeast infections based on a susceptibility survey. Fluconazole is popular due to its administration flexibility, however organisms' resistance to this drug appears to be increasing.[23] Ketoconazole is not recommended in pregnant animals.

Iodine

Addition of povidone-iodine to infusions or uterine lavage solutions is common for management of microbial infections and is included here for completion rather than an update. One study found that intrauterine infusion of 1% povidone-iodine solution in mares causes chronic inflammatory changes in the endometrium.[24] A concentration of 0.5% povidone-iodine still demonstrated suppression of bacterial growth and is a commonly recommended 'safer' dose.[25]

Chelators

Chelators increase the permeability of the bacterial cell wall and cell membrane and are synergistic with antimicrobials including gentamicin, penicillin, oxytetracycline and chloramphenicol. In general, first and second generation chelators have a greater application in treating gram-negative infections. The third generation chelator, Tricide®, appears to potentiate the effects of antimicrobials against gram-positive and gram-negative bacteria as well as yeast and fungi.[26]

Topical antimicrobials

Topical antimicrobials are important components of therapy for vaginal, vestibular and vulvar inflammation and necrosis as most commonly occurs post-foaling. Additionally, the clitoris can occasionally be found to harbor pathogenic organisms and maintenance of the mare's uterine health may benefit from cleansing the clitoris. Ointments that have anecdotal utility include nystatin, neomycin sulfate, thiostrepton and triamcinolone acetonide ointment (Animax®), bovine intramammary treatments (cephapirin), and sodium hypochlorite hydrogel (Anasept®).

Diagnostic methods for identifying infection

The use of intrauterine or systemic antimicrobials for endometritis, the most common mare reproductive tract infection, is ideally preceded by organism identification and antimicrobial sensitivity. Methods for diagnosing endometritis include a uterine swab for microbial culture, endometrial cytology to evaluate for the presence of inflammation, and an endometrial biopsy for histopathology and/or microbial culture. In a comparison of the uterine swab versus endometrial biopsy for assessment of any bacterial growth, one study found that in 2% of all positive cultures, bacterial growth was observed from the swab and not from the biopsy, while in 55% of positive cultures the swab was negative for bacterial growth and the biopsy positive. Calculation of the sensitivity and specificity was performed in two scenarios: culture using a swab compared to culture from an endometrial biopsy as the gold standard, and culture from either a swab or a biopsy compared to inflammatory cells seen in the biopsy as the gold standard.[27]

Swab with culture from biopsy as gold standard:

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Swab culture	0.44	0.98	0.95	0.74

Tests with inflammatory cells in the biopsy as the gold standard[3]:

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Swab culture	0.34	1.00	1.00	0.44
Cytology	0.77	1.00	1.00	0.62
Biopsy culture	0.82	0.92	0.97	0.67

In an assessment of subclinical endometritis as defined by polymorphonuclear cells (PMNs) present during diestrus in the stratum compactum of the endometrial biopsy in the absence of intrauterine fluid, another study compared the value of a uterine swab, a cytology brush, and an endometrial biopsy for cytological and bacteriological diagnosis of endometritis, with the following results:[28]

	Sensitivity		Specificity	
	Cytology	Bacteriology	Cytology	Bacteriology
Swab	0.00	0.33	0.93	0.83
Brush	0.17	0.25	0.83	0.80
Biopsy	0.25	0.25	0.85	0.95
Brush: cytology+bacteriology	0.42		0.70	

These studies suggest that a uterine swab for microbial culture used alone is insufficient for diagnosis of endometritis and that diagnostic ability is improved with addition of at least endometrial cytology. Thus, if bacterial infection is suspected as an underlying cause of the inflammation, antimicrobial treatment may be empirical.

Bacterial growth states

It has been suggested that bacteria are able to survive for long periods in a "dormant" or "viable but non-culturable (VBNC)" state during which time neither plating onto solid media nor inoculation into liquid media lead to growth of cells. Furthermore, bacteria in dormant states are resistant to antimicrobial therapy. Testing for the presence of these bacteria requires fluorescence microscopy and fluorescence labeled antibodies, propidium iodide in conjunction with molecular probes, or amplification of bacterial mRNA using RT-PCR techniques. Some species of bacteria retain their pathogenic potential during dormancy. A human example of the medical importance of non-growth states is *Mycobacterium tuberculosis.*[29-31] Research is actively targeting signals for bacteria to resume growth. An example of such a signal is the resuscitation-promoting factor (Rpf), an enzyme that has been shown to increase the culturability of dormant bacteria. A mechanism by which this enzyme functions in conjunction with other bacterial proteins is under investigation.[32]

In a study to evaluate for a similar bacterial dormancy state in the equine endometrium, Petersen et al used fluorescence in situ hybridization (FISH) to evaluate *S. zooepidemicus* endometritis in mares treated with systemic antibiotics. Despite systemic antibiotic treatment, streptococci could still be visualized deep within the endometrium.[33] Research into the effect of a proprietary "activation" signal for resumption of bacterial growth is ongoing.[34]

Post-mating induced endometritis

Treatment of post-mating induced endometritis in the form of routine antimicrobial infusion post-breeding is common in the Thoroughbred breeding industry. As in any healthy debate, there are at least two sides to the argument. On one hand, there is limited published, statistically significant evidence supporting this practice.[35] Copious use of antimicrobials is also scrutinized for potential antimicrobial resistance development. On the other hand, a study evaluating Thoroughbred reproductive efficiency and financial value found that mares that were barren twice over a seven-year investment were not profitable. This taken together with drift (in the same study, drift was 13.4 +/- 23.2 days) demonstrates that the average mare needs to become pregnant within one to two estrous cycles every year.[36] This may drive the broad treatment for even potential post-breeding infectious endometritis. In a recent study of *S. zooepidemicus* from horses conducted by the University of Kentucky Veterinary Diagnostic Laboratory, resistance of *S. zooepidemicus* has not developed.[37]

Placentitis

Thirty-four percent of equine abortions or stillbirths were associated with feto-placental infection. Of these, 17.8% had an identified bacterial etiology.[38] Antimicrobials are a critical component of bacterial placentitis treatment. Antimicrobials that have evidence of reaching allantoic fluid include penicillin G (22,000 units/kg IM BID), gentamicin (6.6 mg/kg IV SID) and trimethoprim sulfamethoxazole (15-30 mg/kg PO BID).[39,40] Other antimicrobials are anecdotally used with effect. In an experimental ascending placentitis model with beta-Streptococcus, the introduced organism was be cultured from the uterus post-foaling in both treated and untreated controls. The treated ponies received antimicrobials (trimethoprim sulfamethoxazole 30 mg/kg PO BID) continuously until parturition.[41] This information suggests that antimicrobial therapy in pregnant mares with placentitis may need to be prolonged and weighed against side effects of long-term antimicrobial therapy.

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Table 1. Antimicrobial routes and doses

Antimicrobial	Systemic Dose	Intrauterine Dose	
Procaine penicillin G	22,000 IU/kg	5 million IU	
Potassium penicillin	22,000 IU/kg	5 million IU	
Ampicillin	20-40 mg/kg IV TID-QID	3 g	
Ticarcillin and clavulanic acid	50-60 mg/kg IV TID-QID	3.1-6.2 g	0.5 to 1.5 mg/mL in Inra96
Ceftiofur sodium	2.2 mg/kg IV/IM SID-BID	1 g	Marketed as Naxcel in US
Ceftiofur crystalline free acid	6.6 mg/kg IM 2 doses 4 days apart		Marketed as Excede in US
Ceftiofur hydrochloride		1 g	Marketed as Excenel in US
Polymyxin B	6000 U/kg IV BID-TID (antiendotoxic)	1 million U	
Amikacin	10-15 mg/kg IV SID (adults)	2 g	
Gentamicin	6.6 mg/kg IV SID	500-2000 mg	Buffered or diluted
Oxytetracycline	6.6-10 mg/kg SID-BID *slow dilute* IV	(6 g)*	*pending further study
Doxycycline	10 mg/kg PO BID		
Enrofloxacin	6 mg/kg IV SID		
Ciprofloxacin	2.5 g PO SID with 1 g Probenecid PO SID		
Amphotericin B	0.3-0.5 mg/kg IV EOD	100-200 mg	
Nystatin		0.5-2.5 million U	
Ketoconazole	10 mg/kg in 0.2N HCl NGtube BID		
Clotrimazole		500-700 mg	
Miconazole		400-700 mg	
Itraconazole	6 mg/kg PO SID		
Fluconazole	5 mg/kg PO SID	100 mg	

Table 2. Sensitivity of uterine organisms isolated at Hagyard Labortory in 2012. Results are based on cultures collected by Hagyard veterinarians in Central Kentucky. Total number of endometrial cultures=6062. * - Antibiotic not recommended for use with this organism per Clinical Laboratory Standards Institute (CLSI) Guidelines. Penicillin susceptible beta Streptococcus species can be considered susceptible to ampicillin, cefazolin and imipenem per CLSI Guidelines. Of the 6062 cultures, 30 yeast species and 17 fungal species were identified.

UTERINE	beta Strept species (377 iso'd)	Escherichia coli (294 iso'd)	Pseudomonas aeruginosa (100 iso'd)	Staphylococcus aureus (45 iso'd)	Pseudomonas putida (44 iso'd)	Klebsiella pneumoniae (37 iso'd)	alpha Strept species (30 iso'd)	Enterococcus faecalis (27 iso'd)	Enterobacter cloacae (26 iso'd)	Actinobacillus equuli (17 iso'd)	Enterobacter aerogenes (14 iso'd)	Acinetobacter baumannii (13 iso'd)	Citrobacter koseri (12 iso'd)	Nocardia species (8 iso'd)	Pasteurella haemolytica (8 iso'd)	Stenotrophomonas maltophilia (8 iso'd)	Staphylococcus aureus,MRSA (5iso'd)	Acinetobacter lwoffi (5 iso'd)	Flavimonas oryzihabitans (5 iso'd)	Proteus mirabilis (2 iso'd)	Pantoea agglomerans (2 iso'd)	Citrobacter freundii (1 iso'd)
Amikacin	*	96	98	100	100	81	*	*	77	76	93	92	75	88	88	88	80	80	100	100	100	100
Ampicillin	100	41	0	22	2	0	92	100	8	82	0	8	0	75	100	0	0	80	20	0	50	0
Ciprofloxacin	*	94	100	96	93	81	71	56	96	100	100	100	100	88	100	100	100	80	100	100	100	100
Chloramphenicol	99	76	0	100	2	30	94	44	65	100	86	8	75	*	100	38	100	100	40	50	50	0
Gentamicin	*	71	74	73	57	43	53	*	46	88	57	92	17	75	88	13	0	100	100	0	100	0
Imipenem	100	100	100	100	100	100	94	100	100	100	100	100	100	100	100	25	0	100	100	100	100	100
Naxcel	100	93	0	100	9	59	92	*	46	100	71	8	83	88	100	50	0	100	60	100	100	0
Nitrofurantoin	100	98	0	100	0	73	78	100	73	100	93	8	100	25	100	0	100	20	20	50	100	100
Penicillin G	100	*	*	22	*	*	88	96	*	*	*	*	*	*	*	*	0	*	*	*	*	*
Polymyxin B	*	99	100	16	100	100	61	*	100	94	100	100	100	*	88	88	0	60	100	50	100	100
Timentin	100	82	91	100	16	46	*	32	46	94	21	69	33	50	100	88	0	100	40	100	100	0
SXT	96	32	0	49	9	14	35	*	38	53	64	62	8	75	75	25	0	80	60	50	50	100
Doxycycline	73	64	3	73	76	30	75	17	69	94	69	75	75	63	100	100	20	100	80	50	100	0
Tetracycline	60			71			59	0						75			20					