Intrauterine therapies in mares – a clinically applicable review

S.H. Cheong
Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca NY

Introduction

Intrauterine infusions in mares are commonly used by veterinarians to treat a variety of conditions. The intrauterine route offers some advantages over systemic treatments such as the ability to achieve higher concentration of drugs or compounds than is possible through systemic administration, the ability to use potentially toxic substances such as disinfectants, and allow direct contact of the compound to the endometrium. Conversely, the disadvantages of intrauterine infusions include the risk of irritation, contamination, poor retention, and deactivation by uterine content. The choice to include intrauterine infusions can be complicated by the increasing number of compounds and products that have been proposed. This paper summarizes some of the established groups of compounds used as intrauterine treatments and discusses emerging compounds that have been proposed but not yet used in routine practice.

Antibiotics

Bacterial endometritis is the most common reason for intrauterine treatment and it is not surprising that antibiotics are the most established group of compounds used as intrauterine infusion in mares. Antibiotic resistance is a major concern in veterinary and human medicine and judicious use of antibiotics is essential. It is recommended that antibiotic use be based on bacterial culture and sensitivity results or based on morphological appearance of the bacteria on cytological examination. Further, intrauterine antibiotic treatment should be avoided during diestrus as it may promote fungal endometritis. The main concern for intrauterine infusion in mares is the potential irritation to the endometrium from antibiotics. Recommendations are available for the buffering or dilution of some antibiotics to minimize endometrial irritation. Biological material in the intrauterine fluid may reduce efficacy of antibiotics and thus uterine lavage to remove excess biological material in the uterus is recommended prior to intrauterine infusion of antibiotics. Some bacteria may form biofilm that protects the bacteria against antibiotics. Thus, treating mares with suspected biofilm producing bacteria with biofilm reducing agents prior to intrauterine antibiotic infusion will improve efficacy of the antibiotics.

Aminoglycoside

Amikacin sulfate. Amiglyde-V®[a] is licensed for intrauterine use in mares at the dose of 2g (8mL) daily for three consecutive days diluted in 200 mL sterile saline for treatment of endometritis, metritis, and pyometra in mares. Alternatively, amikacin can be buffered by adding at least equal volumes of 8.4% sodium bicarbonate[b] to reduce endometrial irritation. It should not be used on in horses intended for human consumption. Amikacin has been demonstrated to be effective clinically in mares against *Escherichia coli*, *Klebsiella* sp., and *Pseudomonas* sp. In addition, a large number of pathogenic bacteria especially gram-negative bacteria have been shown to be susceptible against amikacin in vitro but its effectiveness may be poorer for gram-positive bacteria. It is not recommended to infuse amikacin on the day of breeding if possible due to the potential detrimental effect on sperm.

Gentamycin sulfate. Sparhawk Laboratories Inc., obtained FDA approval for generic gentamycin sulfate solution use for intrauterine infusion for the treatment of bacterial metritis and improving fertility of mares. It is not to be used in horses intended for human consumption. The recommended dose for intrauterine infusion is 2.0 – 2.5g (20 to 25mL) diluted with 200 – 500mL of sterile saline daily for theee to five days during estrus. The large volume recommended is not easy to retain in the uterus and an alternative method is to buffer gentamycin with a minimum of equal volume of 8.4% sodium bicarbonate[b]. Spectrum of efficacy is better for Gram-negative bacteria and it is not recommended to infuse gentamycin on the day of breeding for the potential impairment of sperm.

Cephalosporin

Ceftiofur sodium. Naxcel®[c] is not labeled for intrauterine use in mares. Several papers have recommended the dose of 1 g diluted in 100 mL sterile saline.[1,2] Spectrum of efficacy includes gram-positive and gram-negative bacteria.

Fluoroquinolone

Enrofloxacin. Is not labeled for intrauterine use in mares. The injectable preparation of enrofloxacin results in severe endometrial inflammation when infused into the uterus at 2.5mg/kg on three consecutive days and is not advisable for intrauterine treatment in mares.[3] More recently, a compounded water-based preparation of enrofloxacin[d] has been evaluated for intrauterine use at a dose of 50mL of 2.5% suspension and has been shown to be safe and did not induce worsening endometrial biopsy score.[4] Spectrum of efficacy includes gram-positive and gram-negative bacteria.

Ciprofloxacin. Is not labeled for intrauterine use in mares. The use of the injectable preparation of ciprofloxacin[e] for intrauterine treatment 60mL of 10mg/mL preparation in mares resulted in seven of ten mares with normal uterine cytology after 24 hours while the remaining two mares had mild inflammation and one mare had moderate inflammation on cytology.[5] While the endometrial response to ciprofloxacin is milder than the injectable form of enrofloxacin, it is not yet determined if the long-term detrimental effects seen for enrofloxacin will occur in mares treated with intrauterine ciprofloxacin. Spectrum of efficacy includes gram-positive and gram-negative bacteria.

Penicillin

Ampicillin. Is not labeled for intrauterine use in mares. Ampicillin[f] 2g diluted in at least 60mL of sterile saline has been proposed but a lower concentration (i.e., more dilute preparation) is recommended as higher concentrations can be irritating to the endometrium.[6] Spectrum of efficacy includes gram-positive bacteria and susceptible *Escherichia coli*.

Ticarcillin with or without clavulanate potassium. The commercial ticarcillin disodium with clavulanate potassium (Timentin®)[g] has been voluntarily withdrawn by the manufacturer and is no longer available. Recommendation of use was 3 to 6g reconstituted in a larger volume of at least 150 to 200mL of sterile saline and is effective against gram-positive bacteria and *Pseudomonas*.

Potassium penicillin. Pfizerpen®[h] is not labeled for intrauterine use in mares. The dose of 5 million IU in 100mL of sterile saline has been shown to reduce growth of *Streptococcus equi subspecies zooepidemicus*[7] and the spectrum of efficacy includes gram-positive bacteria.

Procaine penicillin. Is not labeled for intrauterine use in mares. The spectrum of efficacy includes gram-positive bacteria. Procaine penicillin is substantially cheaper than potassium penicillin.

Polymyxin

Polymyxin B. Polymixin B sulfate is not approved for intrauterine infusion in mares. The spectrum of activity includes gram-negative organisms including *Pseudomonas*. The recommended intrauterine dose of polymixin B is 1 million IU diluted in at least 60mL sterile saline.

Antifungal

Fungal endometritis is commonly treated with intrauterine medications. Many practitioners do not submit fungal cultures and sensitivity as there are only a few laboratories that offer antifungal susceptibility testing and the turnaround time can be lengthy. There are no approved antifungal drugs for intrauterine infusion in mares. Most yeast isolates have good susceptibility to antifungal drugs but mold isolates are much more likely to be resistant to antifungal drugs.[8] Therefore a multimodal therapy using antifungal drugs and other treatments is indicated especially for fungal endometritis with mold isolates. The most common antifungal drugs used for intrauterine infusion are: clotrimazole, nystatin, miconazole, fluconazole and amphotericin B in that order.[6] The course of treatment is recommended to be for five to seven days.

Azole – imidazole

Clotrimazole. The recommended dose is 500 to 700mg of clotrimazole diluted in 50mL of sterile saline. Clotrimazole tablets 100mg can be crushed and resuspended in saline and there are compounded preparations available.

Miconazole. The recommended dose of miconazole for intrauterine treatment is 1,200mg ovules insert of Monistat®[i].

Azole – triazole

Fluconazole. Fluconazole tablets does not dissolve easily in water and requires a vehicle usually DMSO to dissolve. Add at least 1mL of DMSO for each 200mg fluconazole tablet and dilute in sterile water to at least 60mL. Compounded fluconazole preparations for intrauterine infusions are also available.

Polyenes

Amphotericin B. The recommended dose for intrauterine treatment with amphotericin B is 100 to 200mg of Fungizone[j] 50mg per vial. Dilute two to four vials of amphotericin B in sterile saline to at least 100mL.

Nystatin. The recommended dose of nystatin for intrauterine treatment is 0.5 million to 2.5 million IU (5g) suspended in 10mL of sterile saline.

Non-specific antifungal

Lufenuron. The recommended dose for lufenuron treatment is two doses of 266mg Program®[k] infused intrauterine and an additional 266mg dose to be applied to the vaginal vault and clitoral area.

Acetic acid. Distilled vinegar at a concentration of 2% of the commercially available product is a popular treatment for fungal endometritis. The actual concentration of acetic acid in household distilled vinegar varies from high 20's to high 30's % thus the actual concentration of acetic acid used for uterine lavage is much lower than 2%. Preparation of vinegar lavage typically is done by adding 20mL of distilled vinegar to 1L of sterile saline. It is unclear if adding distilled vinegar to lactated Ringer's solution which contains buffers will reduce efficacy and potentially reduce endometrial irritation from acidic pH.

Antiseptic

Povidone iodine. Povidone iodine is more commonly used during the postpartum period but also when treating fungal endometritis. Betadine®[l] solution is available in 5% and 10% strength preparations thus it is important to know which preparation is being used to calculate the dilution. In a 10% solution, there is only 1% active iodine thus to prepare a 0.05% povidone iodine solution, add 5mL of 10% solution to 995mL sterile saline.[9] Higher concentrations of povidone iodine may cause irritation to the endometrium and lower doses are recommended.

Hydrogen peroxide. Traditionally recommended to be used at 1% strength or by diluting 20mL of 3% hydrogen peroxide to 60mL with sterile saline. This concentration of hydrogen peroxide has been shown to be able to reduce biofilm biomass and reduce *Escherichia coli* and *Klebsielle pneumoniae* colony forming units.[10] The same group has recently recommended infusion of 60 to 120mL of 3% hydrogen peroxide for treatment of fungal endometritis.[11]

Dimethyl sulfoxide. Dimethyl sulfoxide (DMSO) is indicated for the reduction of uterine inflammation and to reduce biofilm. Dilute 50 to 200mL of DMSO in 1L sterile saline followed by uterine lavage with sterile saline or lactated Ringer's solution without DMSO.

Tricide® and Tris EDTA. Chelators has been shown to enhance antibiotic efficacy and proposed to disrupt biofilm. The recommendation of use is to infuse 250 to 500mL of Tricide®[m] into the uterus and lavage the uterus with lactated Ringer's solution to remove the debris.

N-acetylcysteine. N-acetylcysteine is a known mucolytic and is effective to treat endometritis from biofilm producing organisms.[10] Dilute 30mL of acetylcysteine (20% or 200mg/mL) into 150mL of

sterile saline and infuse into the uterus. The uterus should be lavaged to recover the uterine content 12 to 24 hours after infusion. The efflux fluid content should be examined for the presence of evidence of mucus and biofilm and repeat treatment as necessary.

Ceragenins. Ceragyn™[n] products contains Purifect™ technology molecules that disrupt bacterial cell membrane. There are two Ceragyn products the Ceragyn lavage and Ceragyn infusion. Ceragyn lavage is diluted in sterile lactated Ringer's solution and can be used to lavage mares 24 hours before and 12 hours after insemination.

Kerosene. Kerosene is a very controversial compound used for intrauterine infusion. The common indication for use is typically a barren mare that has poor uterine biopsy grade even grade III. The subsequent fertility results of previously barren mares are surprisingly good.[1,12] Some care should be observed when performing kerosene intrauterine infusion. First, the equipment used should not contain rubber or weak plastics. Second, the mare should be kept outdoors where the kerosene that may be discharged from the vagina will not cause fires. The infusion volume is typically between 90mL and 500mL and the kerosene should be removed by uterine lavage the following day.

Mycobacterium cell wall immunostimulant. The indication of using Settle™[o] mycobacterium cell wall fraction immunostimulant is to enhance immune function of the mare to overcome endometritis caused by *Streptococcus equi* subspecies *zooepidemicus.*[13] Intrauterine infusion dose is 1.5mL of Settle™ diluted in sterile lactated Ringer's solution to a volume of 25 to 50mL.

bActivate. Latent bacterial infections evade detection and action of the immune system and antibiotics by remaining dormant. bActivate[p] is a compound that reactivates these dormant bacteria making them more susceptible to treatment.[14] The indication of bActivate is to treat mares suspected to have latent bacterial infection. It is recommended to collect a pre-infusion sample for culture by biopsy or low-volume lavage in early estrus. Infuse 10mL of bActivate and collect a repeat culture sample 24 hours after infusion and treat for bacterial endometritis.

Regenerative medicine

Currently, intrauterine use of autologous or allogenic biologics in equine practice is not commonplace. Several groups have begun testing these methods and the results are promising. Most of these products are theorized to regulate the immune response of the uterus to infection and contamination.

Autologous plasma

Autologous plasma is the use of plasma collected from the mare, processed, and then infused into the uterus to moderate the local immune response. Intrauterine infusion with autologous plasma reduced the number of neutrophils after *Streptococcus equip* subspecies *zooepidemicus* inoculation but did not reduce the growth of the bacteria.[7] Autologous plasma intrauterine infusion after breeding was also beneficial for pregnancy rates.[15] Overall the results are encouraging but much more work has to be done to convince the industry to adopt this treatment.

Allogeneic plasma

Allogenic plasma is the use of frozen plasma usually meant for foals with failure of passive transfer to be infused into the uterus. This reduces the work required to produce the plasma for infusion and is likely more appealing to the practitioner.

Platelet-rich plasma

Platelet-rich plasma has been used clinically to modulate inflammatory response in mares for orthopedic conditions but the use in reproduction is still not well established. There are several reports of the beneficial effects of platelet-rich plasma[16-19] but the production of platelet-rich plasma is somewhat difficult in the field and a little costly to use the established systems. The increasing use of platelet-rich plasma in equine orthopedics will likely help the development of this method as more locations are able to produce the product by the sports medicine group in-house and use it in the reproductive unit.

Mesenchymal stem cells

Mesenchymal stem cell isolation technology is constantly being improved and many biotechnology companies are focusing on improving yield and application of bone-marrow derived, adipose derived and umbilical cord blood derived mesenchymal stem cells from horses. The use of allogenic bone-marrow derived mesenchymal stem cells to modulate the immune response to dead-sperm cells[20] and adipose derived mesenchymal stem cells were successfully observed in both uterine horns and body in transplanted mares.[21,22]

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Footnotes

[a] Amiglyde-V®, Zoetis, Kalamazoo, MI
[b] Sodium Bicarbonate Injection 8.4%, Hospira Inc., Lake Forest, IL
[c] Naxcel®, Zoetis, Kalamazoo, MI
[d] Enrofloxacin uterine flush, Rood & Riddle Veterinary Pharmacy, Lexington, KY
[e] Ciprofloxacin, Hospira Inc., Lake Forest, IL

[f] Polyflex®, Bohringer Ingleheim Vetmedica, Duluth, GA
[g] Timentin®, GalaxoSmithKline, Durham, NC
[h] Pfizerpen®, Roerig, New York, NY
[i] Monistat®, Insight Pharmaceuticals, Tarrytown, NY
[j] Fungizone, X-GEN pharmaceuticals Inc., Horseheads, NY
[k] Program®, Novartis Animal Health, Surrey, UK
[l] Betadine® Purdue Pharma L.P., Stamford, CT
[m] Tricide®, Medical Molecular Therapeutics, Athens, GA
[n] Ceragyn™, Pureshield Life Sciences LLC, Walnut Creek, CA
[o] Settle™, NovaVive, Athens, GA
[p] bActivate, Bojesen and Petersen Biotech ApS, Copenhagen, Denmark