**Diagnosis and Treatment of Endometritis**

**Common Problems**
- Fluid in uterine lumen
- Persistent mating-induced endometritis (PMIE)
- Bacterial endometritis
- Uterine cysts
- Fibrosis

**Less Common Problems**
- Fungal endometritis
- Pyometra
- Persistent endometrial cups
- Tumor (i.e. leiomyoma)
- Foreign body
- Urine in uterus

**ENDOMETRITIS**

**Definition:**
- Inflammation of the endometrium
  - Infectious
    - Bacterial
    - Fungal
  - Non-infectious
    - Mating-induced
    - Urine
    - Medications
    - Air, etc.

**MATING-INDUCED ENDOMETRITIS**

- All mares develop a transient inflammatory response after breeding
  - Exposure of the uterus to spermatozoa
- PMNs are present in uterus within 30 minutes after breeding
- Inflammation peaks 8 to 12 hours after mating
- Generally resolved by 24 to 36 hours after mating in the normal mare

**MATING-INDUCED ENDOMETRITIS**

- Inflammation may persist in some individual mares
  - Persistent mating-induced endometritis (PMIE)
- Recognized clinically by the presence of echogenic fluid in the uterine lumen the day after breeding

**UTERINE FLUID VOLUME**

<table>
<thead>
<tr>
<th>Code</th>
<th>Amount of Fluid</th>
<th>Volume (depth in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>T</td>
<td>Trace</td>
<td>&lt; 1 cm</td>
</tr>
<tr>
<td>S</td>
<td>Small</td>
<td>1 to 2 cm</td>
</tr>
<tr>
<td>M</td>
<td>Medium</td>
<td>2 to 4 cm</td>
</tr>
<tr>
<td>L</td>
<td>Large</td>
<td>5 to 10 cm</td>
</tr>
<tr>
<td>VL</td>
<td>Very Large</td>
<td>&gt; 10 cm</td>
</tr>
</tbody>
</table>

Colorado State University
**UTERINE FLUID QUALITY**

<table>
<thead>
<tr>
<th>Code</th>
<th>Visual Assessment</th>
<th>Ultrasound Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Clear</td>
<td>Non-echogenic</td>
</tr>
<tr>
<td>3</td>
<td>Slightly Cloudy</td>
<td>Slightly echogenic</td>
</tr>
<tr>
<td>2</td>
<td>Very Cloudy</td>
<td>Moderately echogenic</td>
</tr>
<tr>
<td>1</td>
<td>Thick, Purulent</td>
<td>Very echogenic</td>
</tr>
</tbody>
</table>

Colorado State University

**MATING-INDUCED ENDOMETRITIS**

- PMIE is associated with a reduction in fertility
  - Especially if inflammatory fluid remains after 48 hours
- Not necessarily associated with an infection* (bacterial or fungal)
- Mares may appear normal until bred/inseminated
- Mares with a history of PMIE are at high risk of reoccurrence

**Incidence Rate:**
- 15 % of Thoroughbred mares and 12 % of QH mares develop PMIE after mating
- Increased incidence in older vs. young mares
- Increased incidence in mares with previous history (uterus is ‘sensitized’)
- Increased risk using frozen semen
  - No seminal plasma

**Management Plan:**
- Monitor estrous cycle
- Induce a timed ovulation with hCG or deslorelin
- Breed mare only once*
- Administer corticosteroids at time of breeding
- Lavage uterus 4 to 6 hours after breeding
- Administer oxytocin or prostaglandins to stimulate uterine contractions and aid fluid removal
- Ultrasound mare at end of lavage
  - Confirm absence of fluid in the uterine lumen

**UTERINE LAVAGE ISSUES IN PROBLEM MARES**

- Poor fluid recovery during lavage procedure
  - Most often encountered in older mares
  - Management
    - Re-fill uterus with flush media
    - Deflate cuff on catheter
    - Advance catheter in uterus
    - Insert ultrasound probe per rectum
    - Administer oxytocin
    - Monitor fluid location/recovery

- Poor fluid recovery during lavage procedure
  - Most often encountered in older mares
  - Management
    - Re-fill uterus with flush media
    - Deflate cuff on catheter
    - Advance catheter in uterus
    - Insert ultrasound probe per rectum
    - Administer oxytocin
    - Monitor fluid location/recovery
BACTERIAL ENDOMETRITIS

- One of the most common uterine problems of brood mares
- Results in decreased fertility
- Accurate diagnosis is important for optimal treatment
  - No single diagnostic test is capable of detecting all cases of infectious endometritis
- Therapy should be based on antimicrobial susceptibility test results

Predisposing Factors:
- Poor perineal conformation/tone
- Abnormal cervical anatomy or function
- Decreased uterine clearance
- Trauma
- Breeding (live cover or insemination)
- Poor breeding hygiene
- Other factors

Diagnosis:
- Ultrasonography
- Speculum Exam
- Culture
  - Guarded swab
  - Low volume lavage
- Cytology
- Biopsy
- Other (RT-PCR)

LOW VOLUME LAVAGE

- Infuse 150 mls of 0.9% sterile saline or LRS; recover effluent
- Centrifuge in conical tube(s) at 600 x g for 10 minutes
- Pellet used for culture, cytologic examination or PCR testing

Microbiology: TSA, MacConkey and Chromogenic Agars

- Streptococcus equi subsp. zooepidemicus
- Escherichia coli
- Staphylococcus aureus
- Pseudomonas aeruginosa
- Klebsiella pneumoniae

Vetlab Supply (Spectrum™ Plates)
www.vetlab.com
**Endometrial Cytology - Neutrophils**

40x Power

**Endometrial Cytology – Bacteria (Cocci)**

Streptococcus equi subsp. zooepidemicus

**Endometrial Cytology – Bacteria (Rod-shaped)**

Escherichia coli

**Bacterial Endometritis: Endometrial Cytology**

**Interpretation:**

<table>
<thead>
<tr>
<th># WBC/hpf*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No inflammation</td>
</tr>
<tr>
<td>1 to 2</td>
<td>Mild inflammation</td>
</tr>
<tr>
<td>3 to 5</td>
<td>Moderate inflammation</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>Severe inflammation</td>
</tr>
</tbody>
</table>

* Number of white blood cells per high power field (x400)

**Most common bacteria:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>% of Positive Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus</em> - beta-hemolytic</td>
<td>56.4 %</td>
</tr>
<tr>
<td>(i.e. Strep. equi subsp. zooepidemicus)</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>14.7 %</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9.9 %</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>0.3 %</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.2 %</td>
</tr>
</tbody>
</table>

Ricketts, 2011

---

**Bacterial Endometritis**

**Treatment:**

- Correct predisposing factors
- Uterine lavage
- ECBolic drugs
  - Oxytocin, prostaglandins
- Intrauterine antibiotics
- Systemic antibiotics (±)
- Other (Tris-EDTA, DMSO, etc.)
- Biofilm treatment
- Caslick (as needed)
Antimicrobial Agents: Intrauterine Administration

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage, Route, Frequency</th>
<th>Antimicrobial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin sulfate (Amiglyde®)</td>
<td>1 to 2 grams, buffer with 10 to 20 mls sodium bicarbonate (8.4 %)</td>
<td>1 to 2 grams</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1 gram</td>
<td></td>
</tr>
<tr>
<td>Ceftiofur (Naxcel®)</td>
<td>1 gram</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500 milligrams</td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin* Water soluble</td>
<td>1,250 mg</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 to 2 grams</td>
<td></td>
</tr>
<tr>
<td>Penicillin (Potassium)</td>
<td>5 million units</td>
<td></td>
</tr>
<tr>
<td>Penicillin (Procaine)</td>
<td>16 mls</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin/Clavulanic acid (Timentin®)</td>
<td>3.1 grams</td>
<td></td>
</tr>
</tbody>
</table>

*Reconstituted into ~ 60 mlis diluent (i.e. sterile water or saline)
*Infuse into the uterine lumen; may repeat for 1 to 3 days or more

Antimicrobial Agents: Systemic Administration

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage, Route, Frequency</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftiofur Crystalline Free Acid (Excede®) (200 mg/ml)</td>
<td>6.6 mg/kg IM</td>
<td>Antibiotic (broad spectrum)</td>
</tr>
<tr>
<td>Enrofloxacin (Baytril®) (50 or 100 mg/ml)</td>
<td>5 mg/kg, IV, q 24 hours or 7.5 mg/kg, PO, q 24h</td>
<td>Antibiotic (broad spectrum) should not be infused into the uterus</td>
</tr>
<tr>
<td>Gentamicin (100 mg/ml)</td>
<td>6.6 mg/kg, IV, q 24h</td>
<td>Antibiotic (Gram-negative spectrum)</td>
</tr>
<tr>
<td>Penicillin (Procaine) (300,000 units per ml)</td>
<td>22,000 IU/kg, IM, q 12h</td>
<td>Antibiotic (Gram-positive spectrum)</td>
</tr>
<tr>
<td>Trimethoprim Sulfamethoxazole</td>
<td>30 mg/kg, PO, q 12h</td>
<td>Antibiotic (broad spectrum)</td>
</tr>
<tr>
<td>Trimethoprim sulfadiazene</td>
<td>24 mg/kg, PO, q 12h</td>
<td>Antibiotic (broad spectrum)</td>
</tr>
</tbody>
</table>

BACTERIAL ENDOMETRITIS

Systemic Antibiotics:

- May be beneficial in addition to or in lieu of intrauterine infusion of antibiotics
- Some antibiotics can be given once every 4 days
  - Excede®- Ceftiofur crystalline free acid (CCFA) 6.6 mg/kg, i.m.
  - Some should not be infused into the uterus
    - Enrofloxacin 5.5 mg/kg, i.v., q24h
  - Other options
    - Trimethoprim-sulfonamide 30 mg/kg, PO, q12h
    - Penicillin (procaine or potassium) 25,000 IU/kg
    - Gentamicin 6.8 mg/kg, i.v., q24h

BIOFILM

- Bacteria can live in a free-floating form within the uterus (i.e. planktonic) or aggregate in a community adherent to the endometrium (i.e. biofilm)
- Bacteria in a biofilm are protected from the host immune system and from antibiotics

Best Fixative To View A Biofilm

<table>
<thead>
<tr>
<th>Fixative</th>
<th>Adherent Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin</td>
<td>0 of 18 samples positive</td>
</tr>
<tr>
<td>Bouin's</td>
<td>18 of 18 samples positive</td>
</tr>
</tbody>
</table>

Biofilm Associated Infections

- Goal is to disrupt the biofilm matrix allowing bacteria to be exposed to antibiotics
- Antibiotics are often not effective as bacteria in a biofilm state are 10x to 1000x* more resistant
- Example:
  - Pseudomonas aeruginosa - Amikacin
    - Planktonic MIC 2 µg/ml
    - Biofilm MIC >16 µg/ml
**FUNGAL ENDOMETRITIS**

- Fungal endometritis is an important, but uncommon, cause of subfertility in the mare
- Incidence rate of 1 to 5% of all cases of infectious endometritis
- Fungal endometritis can be difficult to diagnose and treat

**BACTERIAL ENDOMETRITIS**

- Recurrent or Chronic Infections:
  - Nidus of infection still remaining (biofilm)
  - ‘Dormant’ infections
    - S. zooepidemicus located deep within endometrial tissues
    - May become ‘activated’ and lead to persistent infection
  - Anatomical issues (i.e. perineum, vestibulovaginal seal, cervix)
  - Poor breeding hygiene

**FUNGAL ENDOMETRITIS**

- A wide variety of fungal organisms have been cultured from the mare uterus
- Majority are opportunistic in nature

<table>
<thead>
<tr>
<th>Incidence Rate of Fungal Organisms Isolated by Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>58%</td>
</tr>
<tr>
<td>25%</td>
</tr>
<tr>
<td>12%</td>
</tr>
</tbody>
</table>

Coutinho da Silva and Alvarenga, 2011
**Fungal Endometritis**

**Diagnosis:**
- Uterine culture
  - Guarded swab
  - Low volume lavage
  - Uterine biopsy
- Uterine cytology
  - Swab
  - Cytology brush
  - Low volume lavage
- Molecular techniques
  - RT-PCR

**Yeast**
- Oval to spherical in shape
- 4 μm in diameter
- Thick capsule (‘halo’) that does not take up stain

**Therapy:**
- Uterine lavage or infusion
  - Sterile saline
  - Vinegar in saline (20 mls per liter)
  - Dilute povidone-iodine (10 to 15 mls per liter)
  - Hydrogen peroxide in saline (60 to 120 mls)
- Intrauterine antifungal agents
- Systemic antifungal agents
- Cleanse clitoral fossa and sinuses

**Fungal Endometritis - Cytology**

**Hyphate/Mold (i.e. Aspergillus sp.)**
- Hyphate fungi are cylindrical, thread-like structures several mm to cm in length often with a branching pattern
- Clearly visualized cell walls
- 2 to 10 μm in diameter

**Antimicrobial Susceptibility Tests:**
- Cornell University
  - Animal Health Diagnostic Center
    - Fungal Susceptibility Test
- University of Texas at San Antonio
  - Fungal Testing Laboratory
  - Susceptibility Testing

**Fungal Endometritis - Cytology**

**Antimicrobial Susceptibility Tests:**
- Cornell University
  - Animal Health Diagnostic Center
    - Fungal Susceptibility Test
- University of Texas at San Antonio
  - Fungal Testing Laboratory
  - Susceptibility Testing

**Therapy:**
- Intrauterine antifungal agents
  - Nystatin
    - 5 grams suspended in 50 mls sterile water (x 5 days)
- Systemic antifungal agents
  - Fluconazole
    - 14 mg/kg, PO, loading dose
    - 5 mg/kg, PO, q 24 hours for 2 to 3 weeks
- Caslick as needed
### FUNGAL ENDOMETRITIS

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B*</td>
<td>100 to 200 mg SID</td>
</tr>
<tr>
<td>Clotrimazole*</td>
<td>500 to 700 mg</td>
</tr>
<tr>
<td>Fluconazole*</td>
<td>100 mg</td>
</tr>
<tr>
<td>Miconazole*</td>
<td>200 mg daily or use 1200 mg insert</td>
</tr>
<tr>
<td>Nystatin*</td>
<td>0.5 to 2.5 million units</td>
</tr>
<tr>
<td>Lufenuron</td>
<td>3 to 4 packets (270 mg each)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>6 mg/kg, PO, q 12 to 24 hours</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>14 mg/kg, PO, once (day 1), then 5 mg/kg for additional 9 days</td>
</tr>
</tbody>
</table>

* Reconstituted into 60 to 250 mls sterile saline or water (IU)

### Take Home Message(s):
1. Perform a complete reproductive evaluation on problem mares
2. Consider setting up a microbiology lab in your clinic
3. Select antibiotics based on an antimicrobial susceptibility test
4. Consider Tris-EDTA in combination with antibiotics for treatment of bacterial endometritis
5. Treat fungal endometritis aggressively
6. Manage PMIE with early lavage, oxytocin, etc.

### Follow-up Examination:
- Challenging to treat (successfully)
- Recurrence rate is high
  - Incomplete elimination of organisms
  - Reinfection (source ?)
- Re-culture uterus as needed
- May eliminate fungal organisms and subsequently culture bacterial organism(s)
  - i.e. *Streptococcus equi* subsp. *zoopneumoniae*