Postpartum Uterine Disease in Dairy Cattle
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Physiology vs. pathology
• Almost all cows have bacterial contamination of the uterus after calving
• The same immune system mediators appear to be involved in healthy and affected cows
  – TLR4; IL1, 6, 8, 10; (TNFα)
• Difference lies in the magnitude, regulation, duration and effectiveness of the response

Risk factors for uterine disease
- Species of bacteria
- Virulence factors
- Strain
- Level of contamination

Bacteria Immune response Regulation of inflammation

The uterus is not sterile in virgin heifers or pregnancy
- 10 virgin heifers in estrus and 5 pregnant cows (3rd trimester; slaughter samples)

Uterine E coli
Are the E coli associated with metritis and endometritis specific uterine pathogens or do they have specific virulence factors?
1. Silva et al 2009 – 72 isolates from 12 healthy cows and 18 with metritis – no associations of phylogenetic group or specific virulence factors
  – Strain associations with herd
2. Sheldon et al 2010 – 114 isolates from 64 cows from 1 herd sampled at weeks 1 – 4
  – Association of 1 group with metritis
  – Strains found in cows with metritis were more adherent and invasive to epithelium and LPS induced greater inflammatory response in culture
3. Bicalho et al 2010
  – 611 E coli isolates from 374 cows in week 1 in 4 herds, screened for 32 virulence genes; subset of 117 cows screened for PVD in week 4
  – 6 virulence genes associated with reproductive tract disease
  – Adherence factor (fimH) was the single strongest predictor of metritis (OR 6)

Vaccination against reproductive tract disease
• Total of 371 heifers from 1 herd vaccinated at ~ 50 and 20 d before calving with vaccine containing whole killed E. coli, F. necrophorum and T. Pyogenes ± FimH, leukotoxin, and pyolysin
• SC vaccination with bacteria ± proteins
  – Decreased metritis incidence
    – 12 to ~4% (Dx only at 6 DIM)
    – 28 to 16% (daily Dx by farm staff)
  – Decreased time to pregnancy
    – 120 to 94 median DIM
  – No effect on clinical endometritis (lavage visual at 35 DIM; 9%) or on prevalence of E. coli or F. necro at 2 or 6 DIM, or T. pyo ay 35 DIM

Moore et al JDS 2017
Machado et al 2014
What impacts does this have on this cow? Can we improve her outcomes?

Metritis – mild or severe affects milk production

Only severe metritis, but both PVD and endometritis impair reproduction

Cost of metritis

- Based on data from 500 cases of metritis from 1 large dairy in CA (Overton & Fetrow)
- Treatment $53 - $109/case
  - Ampicillin vs. ceftiofur assuming equal efficacy; depending on drug and milk discard vs. use for calves
- Culling and death $85/case
  - Parity-specific attributable risks:
    - Culling: 1 - 5%  
    - Death: 0.1 - 6.5 %
- Milk loss $83/case ($18 gross milk price)
  - Culled < 30 DIM (6% of cases) -15 lb/d (Med. Exit 10 DIM)
  - Culled 30 - 60 DIM (4% of cases) -9 lb/d (Med. Exit 42 DIM)
  - Retained > 60 DIM (90% of cases) – 6 lb/d to 110 DIM
- Reproduction $109/case
  - 13 vs. 17.5% 21-d pregnancy rate; cost includes reproduction-associated culls
- $329 - $386 per case

Mechanisms of uterine disease effects on fertility

PVD = Purulent Vaginal Discharge

Metritis treatment

- Case definition = T > 39.5 + fetid discharge
- 1) n = 406 cows (8 large herds) randomized to control, 1.1 or 2.2. mg/kg ceftiofur for 5 d
  - Chenault et al., 2004
- 2) n = 982 cows (15 herds) randomized to 2 doses CCFA (3 d apart) or saline control
  - McLaughlin et al. JDS 2012
- Clinical cure (no fever; no fetid discharge) at day 14:
  - Ceftiofur: 77%  
  - Saline: 62%
  - CCFA: 74%  
  - Saline: 55%
Metritis treatment

- n = 528 cases of fetid discharge (n=312) or metritis (fetid + T ≥ 39.5°C; n=216) from 1 large dairy in Florida; 268 matched healthy cows in comparison group
- Blocked by metritis type, randomly assigned to 11 mg/kg ampicillin or 2.2 mg/kg ceftiofur q 24 h x 5 d
- PreSynch 50 and 64 DIM, bred on estrus after 2nd PGF or 5-d Ovsynch with TAI at 84 DIM
- Among cows with fetid discharge only, 53% developed ≥ 1 day of fever while on antibiotics

Lima et al JDS 2014

Metritis treatment - Clinical cure

- Absence of fever + fetid discharge at day 12 not different ~67%
- No interactions of treatment with metritis type, parity, dystocia

Lima et al JDS 2014

Metritis treatment - Reproductive performance

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Ampicillin</th>
<th>Ceftiofur</th>
<th>Healthy comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic 64 DIM</td>
<td>75%</td>
<td>76%</td>
<td>75%</td>
<td>0.96</td>
</tr>
<tr>
<td>Pregnant 1st AI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 d</td>
<td>29%</td>
<td>29%</td>
<td>32%</td>
<td>0.87</td>
</tr>
<tr>
<td>60 d</td>
<td>28%</td>
<td>28%</td>
<td>31%</td>
<td>0.91</td>
</tr>
<tr>
<td>Loss</td>
<td>6%</td>
<td>6%</td>
<td>11%</td>
<td>0.52</td>
</tr>
</tbody>
</table>

These outcomes not different between fetid discharge and metritis

Lima et al JDS 2014

Metritis treatment - Summary

- Diagnostic criteria inconsistent and not well validated (Sannmann et al 2012)
- Systematic review (Haimerl & Heuwieser 2014) and meta-analysis (Haimerl et al 2017) – 23 studies; narrow set of research questions but overall high quality of evidence
- Cows with ≥ 2 of: T > 39.5; fetid discharge; dullness/off-feed can rationally be treated with systemic antibiotics
- Ceftiofur, ampicillin, or penicillin likely to be similarly efficacious (Smith et al 1998; Drillich et al 2001; Chenault et al 2004; Lima et al 2014; review by Reppert VCNA 2015)
Wait and see?

- 1 herd in Germany; daily metritis detection; Rx = 1 dose of CCFA (Sannmann et al Therio 2013)
- 12 treated at Dx 1-4 DIM
- 19 not treated at Dx 1-4 DIM
  - 12 never treated as no metritis at 5-10 DIM
  - 7 with delayed treatment at 5-10 DIM
- 48 treated at Dx 5-10 DIM
- No difference in fever at 4 d after Dx or clinical outcomes to 28 DIM
  - Ability to apply this likely depends on
    - Presence of complicating factors
    - Sensitivity of detection of worsening clinical condition
    - Heat stress
  - Larger scale study needed

NSAID vs antimicrobial for initial treatment of metritis

- 558 cases of metritis (T ≥ 39.5 and fetid discharge); 6 farms in Germany
- Randomized to ketoprofen or ceftiofur
- If T ≥ 39.5 on day 4-7:
  - KET → 3 d of ceftiofur
  - CEF → + 2 d of ceftiofur

Metritis treatment - Summary

- Expected outcomes:
  - Absence of fever in 80 - 100% of cows 2-6 days after treatment
  - Absence of fetid discharge 33 - 77%
  - No difference in reproductive performance for treated cases of metritis (Dubuc et al 2011; Lima et al 2014)
- More field research is needed to refine treatment selection criteria (i.e. discharge ± temperature), and assess antimicrobial resistance, animal welfare, and economics

Impact of Metritis on Milk Production

- Multiparous (metritis)
  - 350 kg less milk per lactation for multiparous cows with metritis
- Primiparous (metritis)

Cows with metritis produced less milk in the long term

- 250 kg less milk per lactation for multiparous cows with metritis

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 mg/kg ketoprofen</th>
<th>3 mg/kg ceftiofur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended treatmenta (%) (ns)</td>
<td>61 (142/226)</td>
<td>41 (90/220)</td>
</tr>
<tr>
<td>Persistent vaginal discharge (%) (ns)</td>
<td>56 (116/226)</td>
<td>53 (127/220)</td>
</tr>
<tr>
<td>First AI pregnancy (%) (ns)</td>
<td>20.46 (229)</td>
<td>25 (302/223)</td>
</tr>
<tr>
<td>Mean milk yield ± SE</td>
<td>25.5 ± 1.1</td>
<td>20.3 ± 1.1</td>
</tr>
<tr>
<td>Median days to first AI by 200 DIMb</td>
<td>(n = 290)</td>
<td>(n = 290)</td>
</tr>
<tr>
<td>Median days to pregnancy by 200 DIMb</td>
<td>(n = 290)</td>
<td>(n = 290)</td>
</tr>
</tbody>
</table>

Pohl et al JDS 2016

Huzzey et al., 2007

Huzzey et al. 2007

Dubuc et al, 2010

Dubuc et al 2011

Lima et al 2014

Dubuc et al, 2010
Impact of metritis on reproduction & culling

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy risk (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First service</td>
<td>35.3</td>
<td>27.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>120 DIM</td>
<td>46.0</td>
<td>36.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>300 DIM</td>
<td>80.4</td>
<td>82.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Culling risk (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 DIM</td>
<td>3.6</td>
<td>4.5</td>
<td>0.70</td>
</tr>
<tr>
<td>63 DIM</td>
<td>6.8</td>
<td>7.2</td>
<td>0.92</td>
</tr>
<tr>
<td>300 DIM</td>
<td>17.0</td>
<td>18.0</td>
<td>0.83</td>
</tr>
</tbody>
</table>

No full-lactation effects when parity, production, DA, endometritis, pregnancy accounted for

Metritis Treatment

- Diagnostic criteria inconsistent and not well validated (Sannmann et al 2012)
- Cows with ≥ 2 of: T > 39.5; fetid discharge; dullness/off-feed should be treated with systemic antibiotics
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- Expected outcomes:
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  - Absence of fetid discharge 33 - 77%
- More field research is needed to refine early treatment selection criteria

Why do cows have uterine disease?

- Cows with normal uterine health n = 22
- Cows with metritis < 14 DIM n = 18
- Cows with subclinical endometritis at 28 DIM n = 43

Hammon et al, 2006

Neutrophil myeloperoxidase (iodination) activity - a measure of killing activity

- Cows with normal uterine health n = 22
- Cows with metritis < 14 DIM n = 18
- Cows with subclinical endometritis at 28 DIM n = 43

Hammon et al, 2006
Increased Haptoglobin Precedes Reproductive Disease

Hp ≥ 1 g/L at 3 DIM OR = 6.7 for metritis Se = 50% Sp = 87%

Hp > 0.8 g/L in week +1 increased odds of metritis, PVD, and endometritis (OR = 1.6 – 2.2) (Dubuc et al 2010)

Feed intake

Inflammation

• Acute/classical inflammation
  – Injury → heat, pain, swelling, redness
  – Infection → fever, decreased feed intake
  – Disease causes inflammation

• Metabolic/chronic inflammation
  – No clinical signs
  – Oxidative stress, obesity, fat mobilization (?) → Slight elevations in pro-inflammatory mediators and acute phase proteins; changes in signalling
  – Contributes to insulin resistance
  – Inflammation contributes to disease

Adapted from Barry Bradford

Associations with reproductive tract inflammatory disease

Concepts of inflammatory response
Reproductive tract infection and inflammation

Endometritis

PVD

Each affects 15 - 20% of cows

Subclinical endometritis: endometrial cytology

R. Lefebvre, Université de Montréal

Endometritis - common and important

Prevalence of Uterine Disease

Classification of risk for uterine disease

<table>
<thead>
<tr>
<th></th>
<th>Low risk (Dystocia, twins, RP)</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metritis</td>
<td>12%</td>
<td>28%</td>
</tr>
<tr>
<td>EXAM 35 DIM</td>
<td>n=1116</td>
<td>n=956</td>
</tr>
<tr>
<td>Endometritis only</td>
<td>13%</td>
<td>13%</td>
</tr>
<tr>
<td>PVD only</td>
<td>5%</td>
<td>15%</td>
</tr>
<tr>
<td>Both</td>
<td>4%</td>
<td>10%</td>
</tr>
<tr>
<td>Total</td>
<td>22%</td>
<td>38%</td>
</tr>
</tbody>
</table>

Disease and risk

• PVD ≠ endometritis
  – Poor agreement (42% of cows with PVD had concurrent endometritis; Dubuc et al 2010a)
  – Different risk factors (Dubuc et al 2010b)
  – Additive effects on reproduction and pregnancy loss (Dubuc et al 2010; Lima et al 2013)
  – Cervicitis is a co- or independent factor in many cases

Reproductive tract inflammatory disease

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  – Poor agreement (42% of cows with PVD had concurrent endometritis; Dubuc et al 2010a)
  – Different risk factors (Dubuc et al 2010b)
  – Additive effects on reproduction and pregnancy loss (Dubuc et al 2010; Lima et al 2013)
  – Cervicitis is a co- or independent factor in many cases

R. Lefebvre, Université de Montréal
Impact of Reproductive Disease on Time to Pregnancy

Reproductive tract disease change and interval to pregnancy

Cervicitis

Relationships among reproductive tract inflammatory diseases

Inflammation vs. infection

Treatment of PVD and time to pregnancy
Materials & Methods

2072 cows in 6 herds in Ontario, Canada and New York, USA

Disease recording

<table>
<thead>
<tr>
<th>Variable</th>
<th>NEFA</th>
<th>NEFA, BHB, Haptoglobin</th>
<th>Exam for CYTO &amp; CLIN</th>
<th>AI, pregnancy diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-7</td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>28</td>
<td>35</td>
<td>42</td>
<td>49</td>
<td>56</td>
</tr>
<tr>
<td>63</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CCFA High risk n=1048
PGF All cows

P4 NEFA, BCS

PGF Therapy - Clinical Cure

<table>
<thead>
<tr>
<th>Week 5 status</th>
<th>P4</th>
<th>P4</th>
<th>P4</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVD + (ignoring CYTO)</td>
<td>323</td>
<td>72%</td>
<td>58%</td>
<td>.01</td>
</tr>
</tbody>
</table>

- No effect of PGF on cure of cytological endometritis
- Among cows 'clean' at week 5 (n=1432), no effect of PGF on new PVD or CYTO (6 - 8 % incidence)
- No overall effect of PGF on pregnancy rate

Prevalence of CL

Cows with PVD (n = 332)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Cows with serum P4 &gt; 1 ng/ml (%)</td>
<td>23</td>
</tr>
<tr>
<td>Cumulative proportion (%) with ≥ 1 sample with P4 &gt; 1 ng/ml</td>
<td>-</td>
</tr>
<tr>
<td>CL status at PGF</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Once</td>
</tr>
<tr>
<td></td>
<td>Both</td>
</tr>
</tbody>
</table>

PGF treatment of PVD

<table>
<thead>
<tr>
<th>n = 318</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows with PVD at week 5</td>
</tr>
<tr>
<td>Accounting for parity, dystocia/RP/twins, BCS at calving, herd</td>
</tr>
<tr>
<td>2 shots PGF: HR = 1.2 (0.95 – 1.6)</td>
</tr>
<tr>
<td>P = 0.07</td>
</tr>
<tr>
<td>No PGF*CL interaction</td>
</tr>
</tbody>
</table>
Clinical Trial in NZ
756 cows with PVD > 14 DIM
26% with palpable CL

<table>
<thead>
<tr>
<th>Outcome</th>
<th>IU Cephapirin to all</th>
<th>Ce-dependent treatment CL: PGF</th>
<th>No CL: cephapirin</th>
<th>Model P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical cure 14 d</td>
<td>84.1</td>
<td>81.1</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>Submission 21 d</td>
<td>86.4</td>
<td>87.7</td>
<td>.98</td>
<td></td>
</tr>
<tr>
<td>Pregnant 1st service</td>
<td>41.6</td>
<td>36.9</td>
<td>.60</td>
<td></td>
</tr>
<tr>
<td>42 d In Calf</td>
<td>64.3</td>
<td>61.9</td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>Final In Calf (12 wk)</td>
<td>85.8</td>
<td>86.9</td>
<td>.46</td>
<td></td>
</tr>
</tbody>
</table>

Clinical cure 14 d, IU Cephapirin to all; Ce-dependent treatment CL: PGF | No CL: cephapirin, Model P value

Results - Interval from start of mating to pregnancy

HR = 1.00 [0.85 - 1.16] P = 0.96
Median interval to pregnancy
IU-all = 26 d CL-dep = 27 d

PGF treatment

• n = 1342 in 1 herd
• Control: PGF at 25 ± 39 DIM
• All bred by Double-Ovsynch at 75 DIM
• No effect on pregnancy at 32 d (40%) or pregnancy loss to 60 d (12%)
• PGF at 25 d ↓ endometritis (PMN > 5%) at 32 but not 46 DIM
• Only endometritits still present at 46 DIM ↓ pregnancy at 1st AI (to 25%) and ↑ pregnancy loss (to 44%)
• Cows with both PVD at 25 DIM and endometritis at 25, 32, or 46 had ↑ pregnancy loss (to 30%)

Lima et al 2013

PGF treatment of PVD or endometritis
Meta-analysis

• n = 5 trials (6 studies) included
• Endometritis > 21 DIM
  – NB - varied case definitions
• Mean difference in time from calving to pregnancy was not different to favouring control groups
• Heterogeneous results - reasons unclear
  – Possible publication bias

Haimerl et al JDS 2013

Anti-inflammatory therapy for endometritis

• Treatment (Preist et al 2012)
  – 213 cows with > 14% PMN at 14 DIM randomized to carprofen 3X q 3 d starting at 21-25 DIM
  – No effect on PMN% at 42 DIM (92-96% < 14%)
  – Carprofen decreased time to pregnancy among PMN >14% at 14 DIM
  – No effect on production, metabolites, or % anovular at 45 DIM
• Prevention (Meier et al 2014)
  – 659 pastured cows in 2 herds in NZ
  – Carprofen 1.3,5 or 19, 21, 23 DIM
  – No effect on
    • PVD or PMN% at 13-24 or 30-49 DIM
    • Production (c/f Forsey et al 2013 salicylate effects)
    • Metabolites (small ↓ in albumin, BHBA ~ 35 DIM)

Reproductive health monitoring

Metriz – daily screening
Ketosis – (bi-)weekly testing

3 14 21 28 42

Metricheck

Reproductive health monitoring

3

14

21

28

42

Metrichcek
### Transition checklist

**Goal:** Optimize metabolic health & immune function

**Means:** Manage cows to maintain feed intake

<table>
<thead>
<tr>
<th>Management</th>
<th>Transition diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Feed daily for 3-5% left over</td>
<td>- 3-4 weeks on close-up diet or 6 weeks as a dry group</td>
</tr>
<tr>
<td>- ≥ 30&quot; (75 cm) bunk space per cow</td>
<td>- Meet but do not exceed E requirement 8 to 3 weeks prepartum</td>
</tr>
<tr>
<td>- ≤ 85% cows:stalls</td>
<td>- Water ad lib. 10 cm linear per cow, 2 sources per pen</td>
</tr>
<tr>
<td>- ≥ 120 ft$^2$ (11m$^2$) of bedded pack/cow</td>
<td>- 1000 IU vitamin E/d, up to 2000 IU/d for RP; 0.3 ppm selenium, ideally 6 mg/d</td>
</tr>
<tr>
<td>- Build for 130-140% of average monthly calvings</td>
<td>- THI &lt; 68</td>
</tr>
<tr>
<td>- Comfort in stalls; adaptation</td>
<td>- NEFA ≤ 0.4 in last week prepartum, ≤ 1.0 in week 1</td>
</tr>
<tr>
<td>- &lt; 24 h in calving pen</td>
<td>- BHB ≤ 1.1 mM in week 1</td>
</tr>
<tr>
<td>- Separate heifers</td>
<td>- BHB ≤ 1.2 mM weeks 2 - 3</td>
</tr>
<tr>
<td>- Minimize group changes</td>
<td></td>
</tr>
<tr>
<td>- Heat abatement when THI &gt; 68</td>
<td></td>
</tr>
<tr>
<td>- BCS = 3.0 - 3.5 at calving</td>
<td></td>
</tr>
</tbody>
</table>

**Monitoring**

- NEFA ≤ 0.4 in last week prepartum, ≤ 1.0 in week 1
- BHB ≤ 1.1 mM in week 1
- BHB ≤ 1.2 mM weeks 2 - 3