Infection and innate immunity in the female genital tract

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Farmers and veterinarians are rising to the grand challenge to feed the world.
But there is a problem: uterine infections increasingly cause endemic disease and reduce productivity.

Bacterial infection of the uterus after parturition affects all dairy cattle.

40% develop clinical disease.

Animals remain infertile even after successful treatment of the disease.

€1.4 billion/year in EU
$0.6 billion/year in USA
Bacteria cause uterine disease

Complex uterine microbiome

**Widely accepted pathogens**

Gram-negative
*Escherichia coli*

Gram-positive
*Trueperella pyogenes*

Anaerobes
*Prevotella spp*
*Fusobacterium spp*

Griffin et al 1974 *Therio* 1, 91  Dohmen et al 1995 *Therio* 43, 1379
Post partum uterine bacterial infection and disease is common in dairy cattle

Sheldon et al 2009 Biol Reprod 81:1025
The mechanisms of inflammation and defence in the bovine endometrium - a missing component?

1. What evidence is there for the role of innate immunity in the postpartum uterus?
2. What factors might scale the inflammatory response in the endometrium?
3. What impact does uterine infection have on ovarian function?

David Noakes
Innate immunity depends on cellular receptors that bind pathogen-associated molecular patterns.

Nobel Prize in Physiology or Medicine 2011 awarded for discoveries concerning Toll and Toll-like receptors (TLRs) for the activation of innate immunity by pathogen-associated molecular patterns.

Jules Hoffmann

Bruce Beutler
Hematopoietic innate immune cells sense pathogen-associated molecular patterns.

TLR4

- E. coli
- Pathogen clearance
- Attract and activate more macrophages and neutrophils

Inflammatory gene expression

Cytokines IL-1β IL-6 IL-10
Chemokines IL-8 CCL5
Prostaglandins PLA2 PGE₂
Antimicrobial peptides LAP TAP

Macrophage
Neutrophil

CD14 MD2

NFκB ERK p38
Bacterial infection of the uterus causes disease with inflammation and damage of the endometrium.

**Clinical disease**

**Histopathology**

**Gene transcription**

- Innate immunity *TLR4*
- Prostaglandins *PLA2*
- Cytokines *IL1B IL6*
- Chemokines *IL8 CCL5*
- Antimicrobial peptides

Inflammation - neutrophils - macrophages - damage
Evidence for innate immune responses - *in vivo* acute phase protein response

Haptoglobin (µg/mL)

Days postpartum

- Toxic metritis n = 13
- Metritis n = 13
- Normal n = 7

Sheldon & Dobson 2001 *Vet Rec* 148, 172-175
Uterine infection increases the concentrations of LPS, acute phase proteins, and prostaglandin E in blood

Herath et al 2009
Endocrinology 150:1912-1920
Innate immunity in the postpartum endometrium

Herath et al 2009 Reprod Biol Endocrinol 7: 55
Endometrial organ cultures accumulate inflammatory mediators in response to live *E. coli* and *T. pyogenes*

**IL-1β**

Control  | E. coli | T. pyogenes
---|---|---
0 | [High Value] | [High Value] (*p* value)

**IL-6**

Control  | E. coli | T. pyogenes
---|---|---
0 | [High Value] | [High Value] (*p* value)

**IL-8**

Control  | E. coli | T. pyogenes
---|---|---
0 | [High Value] | [High Value] (*p* value)

*Borges et al 2012 AJR 67, 526*
Endometrial cells mount inflammatory response to pathogen-associated molecules

Turner et al 2014 *Endocrinology* 155, 1453
Endometrial cells detect pathogen-associated molecules

0 min 45 min

vehicle

LPS

NFκB

Cronin et al 2012 *Biol Reprod* **86**, 51
Endometrial cells mount inflammatory response to pathogen-associated molecules via TLRs

Primary stromal cells

Cronin et al 2012 *Biol Reprod* 86: 51
Turner et al 2014 *Endocrinology* 155, 1453-1465

si = siRNA
LPS switches prostaglandin secretion in endometrial epithelial cells

Stromal cells  Epithelial cells

Herath et al. 2009 *Endocrinology* 150:1912-1920
Summary 1: Endometrial cells generate inflammatory responses to pathogen-associated molecules.
What factors might scale the inflammatory response in the endometrium?

Danger model

DAMPs
Damage-associated molecular patterns

Polly Matzinger

Healey et al 2014 Scientific Reports 4: 7060
Host tissues and cells respond to IL-1α but not prototypical DAMPs, HMGB1 or hyaluronan.

HA – hyaluronan – extracellular matrix DAMP
HMGB1 – nuclear protein – cellular DAMP
IL-1α - intracellular cytokine DAMP
LPS – pathogen-associated molecule

Healey et al 2014 Scientific Reports 4: 7060
Endometrial cells sense and react to tissue damage during infection via interleukin 1α

**Epithelium**

- **Control** vs. **LPS**
  - **Cell lysate**
    - Control: 0, LPS: 6
  - **Supernatant**
    - Control: ND, LPS: ND

**Supernatant**

- Normal vs. Necrosis
  - **LPS**
    - Normal: ND, Necrosis: ND

**Stroma**

- **Control** vs. **LPS**
  - **Cell lysate**
    - Control: ND, LPS: 30
  - **Supernatant**
    - Control: ND, LPS: ND

**Supernatant**

- Normal vs. Necrosis
  - **LPS**
    - Normal: ND, Necrosis: 40

Healy et al Scientific Reports 2014; 4: 7060
IL-1α scales endometritis when there is infection followed by cell damage

Healey et al 2014 Scientific Reports 4: 7060
Countering infections requires a robust immune response and then resolution of the inflammation. What goes wrong in dairy cattle?
Reducing the availability of glucose blunts the innate immune response

Endometrial organ culture

Turner et al 2016 PLoS ONE 11: e0151416
The sensor of cellular energy - AMPK - regulates the innate immune response

Endometrial organ culture

Turner et al 2016 PLoS ONE 11: e0151416
Summary 2: The innate immune response reflects the severity of challenge and impact of the environment

- Sensing of vita-PAMPs
- Inflammatory responses to PAMPs
- Autocrine and paracrine regulation of innate immune response
- Sensing DAMPs from cell and tissue damage
- Microbial virulence factors disrupt cells
- Phagocytosis and elimination of microbes
- Active resolution of inflammation
- Duration of microbial infection
- Amplitude of inflammatory response

Stern et al. 2016 J Reprod Immunol
What impact does uterine infection have on ovarian function?

- Follicle diameter (mm)
- Oestradiol (pg/ml)
- Follicular Fluid LPS (ng/ml)

Uterine infection

- Normal uterus
- Uterine infection

- Days post partum
- Days post partum

- LPS
- n = 7

- Oestradiol (pg/ml)
- Oestradiol (pg/ml)

- None
- Mild
- Moderate
- Severe

- Follicle diameter (mm)
- Follicle diameter (mm)

- follicular fluid LPS (ng/ml)
- follicular fluid LPS (ng/ml)

Sheldon et al 2002 Reproduction 123, 837
Herath et al 2007 Reproduction 134, 683
Healthy ovarian follicles do not contain immune cells

Bromfield & Sheldon 2011 *Endocrinology* **152**, 5029
LPS impacts ovarian granulosa cell function

**IL-6**

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-6 (pg/ml)</th>
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<tbody>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>siTLR4</td>
<td>500</td>
</tr>
<tr>
<td>LPS</td>
<td>1000</td>
</tr>
<tr>
<td>siTLR4 + LPS</td>
<td>1500</td>
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**Oestradiol**

<table>
<thead>
<tr>
<th>LPS (µg/ml)</th>
<th>Oestradiol (ng/ml)</th>
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<tbody>
<tr>
<td>C</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
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<td>10</td>
<td>12</td>
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**COC IL-6**

<table>
<thead>
<tr>
<th>LPS (µg/ml)</th>
<th>IL-6 pg/COC</th>
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<tbody>
<tr>
<td>0</td>
<td>0.5</td>
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<tr>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>1</td>
<td>2.1</td>
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<tr>
<td>10</td>
<td>3.0</td>
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Bromfield & Sheldon 2011 Endocrinology 152, 5029
Price et al 2013 Endocrinology 154, 3377
Summary 3: Oocytes and ovarian follicles are sensitive to pathogen molecules throughout development

Bromfield & Sheldon 2011 Endocrinology 152, 5029

290 COCs; * P < 0.05
Conclusion: innate immunity is a key mechanism linking infection and infertility in the uterus and ovary.

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