Influence of Experimentally-induced clinical mastitis on Reproductive Performance of Dairy Cattle

Dr. Mitch Hockett
Department of Animal Science
North Carolina State University
Characteristics of Mastitis

- Swelling of the mammary gland
- Decreased milk production
- Abnormal appearance of milk
- Elevated body temperature
- Depression
- Decreased feed consumption
Economic Losses associated with Mastitis

- Decreased milk production
- Antibiotic treatment
- Involuntary culling
Economics of Mastitis

- Decreased milk production
- Antibiotic treatment
- Involuntary culling
- Increase days open
- Increase services per conception
Summary of Previous Work

- Moore and O’Connor (1993) suggest clinical mastitis caused by gram-negative bacteria may result in altered interestrus intervals and cystic ovarian disease.
- Cullor (1990) reported altered interestrus intervals in cattle infused IV with *E. coli* LPS.
Summary of Previous Work

- Peter et al. (1989) observed an increase in plasma cortisol after intrauterine infusions of *E. coli* endotoxin.

- Infusion of *E. coli* endotoxin (IU) resulted in elevated cortisol and decreased estradiol levels in Holstein heifers (Peter et al., 1990).
Summary of Previous Work

Huszenicza et al, 2005

- Observed cows post-partum
- Clinical mastitis between days 14-28 post calving delayed onset of estrus, cyclicity, and ovulation
- Gram-negative pathogens-luteolysis (46%) compared to Gram-positive (8%) and no mastitis (2%)
- During follicular phase-extended by up to 3 days
Summary of Previous Work

- Petersson et al, 2006
- Swedish dairy across 15 years
- Cows treated for mastitis early in lactation had 18 days longer calving to first luteal activity
Previous Work

- Barker et al. (1998)
  - Mastitis prior to breeding
    - Increased days to first service
    - Increased days open

  - Mastitis after first insemination
    - Increased services per conception
    - Increased days open
Effects of Mastitis Type Before First Insemination

- Days to first insemination
- Days open
- Services/conception

Schrick et al., 2001
Effects of Mastitis Type During Breeding Period

Schrick et al., 2001

- Clinical
- Subclinical
- Control

Days to first insemination

- Days

Number of services

- Days open
- Services/conception

Schrick et al., 2001

Effects of Mastitis Type During Breeding Period

Clinical
Subclinical
Control

Days to first insemination

Days open

Services/conception
Gram-positive and Gram-negative pathogens equally decreased reproductive efficiency.

Clinical and subclinical infections equally decreased reproductive parameters.
Retrospective analysis
Need for controlled studies
Utilized challenge model with *Strep uberis*
Mastitis \Rightarrow Pregnancy

? \Rightarrow Days to first service

Days to first service \Rightarrow Services/conception

Mastitis \Downarrow Pregnancy
Study 1: Determine the effect of clinical mastitis induced by Strep uberis on:

- Quarter health
- Body Temperature
- Milk Production
- Somatic Cell Count
- LH peak (following GnRH)
- cortisol concentrations
- PGFM peak (following OT)
Materials and Methods

- 20 Jersey cows (n=20)
  - Group 1: Control (n=10)
  - Group 2: Treated *Strep. uberis* (n=10)

- Intensive collection for serum analysis

- Oxytocin challenge at 2 h, GnRH at 4 h

- Blood samples at infusion and weekly for 5 weeks
## Clinical Observations

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarter 1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quarter 2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rectal Temp&lt;sup&gt;2&lt;/sup&gt;</td>
<td>38.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>38.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.0&lt;sup&gt;cb&lt;/sup&gt;</td>
<td>38.5&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>38.9&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk&lt;sup&gt;3&lt;/sup&gt;</td>
<td>26.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>25.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>20.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>16.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.0&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>15.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SCC&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>6.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Hockett et al., 2000

<sup>1</sup> P<.0001  
<sup>2</sup> P<.05  
<sup>3</sup> P=.03  
<sup>4</sup> P=.0001
Concentrations of LH

![Graph showing concentrations of LH over time with GnRH injection highlighted.]

- LH (ng/ml)
- TIME (hour)
- TRT, CON
- GnRH
LH Results

- Baseline LH higher for CON (2.2 ± 0.1) vs TRT (1.9 ± 0.1 ng/ml; P=.06)
- Peak values of LH tended to be higher for CON cows (26.8 ± 4.1 ng/ml) than TRT cows (15.4 ± 5.8 ng/ml, P=.08)
- Change from baseline to peak tended to be higher for CON (24.6 ± 4.6) than for TRT (12.4 ± 5.8 ng/ml, P=.09)
Concentrations of Cortisol

Cortisol (ng/ml)

Day 0 Day 4 Day 7

TRT

CON

* *

DAY

0 2 4 6 8 10 12 14 16 18

Day 0 Day 4 Day 7
Mastitis ⇓ Pregnancy

Days open

↑ Cortisol

Estrous expression

Concentrations of PGFM

Hockett et al., 2000

(*P=.006)
Mastitis \downarrow \text{Reproduction}

\text{Uterine-Ovarian Axis}

\begin{itemize}
  \item PGF$_{2\alpha}$
  \item Body Temp
  \item Immune Response (−)
  \item Embryo Development
  \item Luteal Length
  \item Pregnancy Rates
\end{itemize}
Prostaglandins and Reproduction

- **Decreased pregnancy rates** (Schrick et al., 1993; Seals et al., 1998; Lemaster et al., 1999)

- **Reduced embryonic development and quality** (Hockett et al., 1998)

- **Decreased embryo development in vitro** (Buuck et al., 1996; Fazio et al., 1997; Scenna et al., 2002)
Study 2 Objective

- To determine the effects of clinical mastitis during the preovulatory time period on expression of estrus and pregnancy rates
Materials and Methods

- Jersey cows 60-90 DIM (n=29, 2 reps)
- Pre-synchronized estrus (d -22, -8)
- Challenged with *S. uberis* (n=14; d2)
- Unchallenged control (n=15, CON)
- Administered PG on d 6
- Observed for estrus and artificially inseminated
Materials and Methods

- Milk samples collected daily (7d) for microbiology and SCC (beginning at challenge)
- Mastitis scores daily (7d)
- Antibiotic treatment 7 d following challenge
**Materials and Methods**

Screening ➔ *Strep. uberis* challenge ➔ Artificial Insemination ➔ Pregnancy Determination

-22 ➔ PGF ➔ -8 ➔ PGF ➔ 0 ➔ PGF ➔ 2 ➔ PGF ➔ 6 ➔ PGF ➔ 9 ➔ PGF ➔ 11 ➔ PGF ➔ 22 ➔ PGF ➔ 35

**Every other day blood samples for BUN, NEFA, insulin, cortisol and P4**
Concentrations of Cortisol

Cortisol concentrations (ng/ml)

Days of experiment

1 3 5 7 9 11 13 15 17 19

Concentrations of Cortisol

Challenge

(* P=.01)
Percentage in Estrus

CON

TRT-PRE

* P< .02
Days to Estrus

*P<.06
Cows with clinical mastitis had:

- Increased mean concentrations of BUN
- Increased mean concentrations of insulin
- Increased concentrations of cortisol
- Increased days to estrus
Hypothalamo-Pituitary-Ovarian Axis

Cortisol
\[\text{(-)}\]

Estrous Expression
Ovulation

Mastitis \(\downarrow\) Pregnancy

\[\text{Feed Intake} \quad \text{Altered Metabolites} \quad \text{Hormonal Alterations} \quad \text{Follicular Development} \quad \text{Pregnancy Rates} \]

Nutritional Interaction
Study 3 Objective

- To determine if experimentally-induced clinical mastitis prior to ovulation results in alterations of endocrine function, follicular growth, and/or ovulation of the preovulatory follicle
Materials and Methods

- **Intensive sampling period (d 6)**
  - Blood samples collected every 15 min (8 h): LH pulsatility
  - Ultrasound performed every 6 h until ovulation: Max LH, E₂
  - Estrous detection every 2 h
Strep. uberis challenge

Intensive blood sampling every 10 min (8h)

Blood sampling (2 h) &
Ultrasonography (6 h)
Results

- Control cows free of clinical mastitis (n=12)
- Challenge cows that developed clinical mastitis prior to estrus expression (n=9)
Estrous Expression

- *P=.01
Two populations exist within experimentally-challenged animals
- Express estrus (n=5; TRT-EST)
- Did not express estrus (n=4; TRT-NOEST)
- Unchallenged cows (n=12; CON)
Clinical Observations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>TRT-EST</th>
<th>TRT-NOEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary scores(^1)</td>
<td>1.0 ± .2 (^a)</td>
<td>3.2 ± .3 (^b)</td>
<td>4.0 ± .3 (^b)</td>
</tr>
<tr>
<td>SCC(^2) ((\log_{10} \text{cells/ml}))</td>
<td>4.6 ± .16 (^c)</td>
<td>5.2 ± .1 (^d)</td>
<td>5.9 ± .1 (^e)</td>
</tr>
<tr>
<td>Rectal Temp(^3) ((^\circ\text{C}))</td>
<td>38.4 ± .2 (^f)</td>
<td>38.5 ± .2 (^f,g)</td>
<td>38.7 ± .2 (^g)</td>
</tr>
</tbody>
</table>

\(^1\) \(^a,b\)(P< 0.0001)
\(^2\) \(^c,d,e\)(P<0.0001)
\(^3\) \(^f,g\)(P=0.02)
## LH Profile and Follicle Size

<table>
<thead>
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<th>TRT-EST</th>
<th>TRT-NOEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH max value(^1) (ng/ml)</td>
<td>11.3 ± 2.0(^a)</td>
<td>7.1 ± 2.6(^{a,b})</td>
<td>1.2 ± 2.9(^b)</td>
</tr>
<tr>
<td>LH pulse frequency(^2) (number/8h)</td>
<td>8.4 ± 0.4(^a)</td>
<td>6.8 ± 0.4(^b)</td>
<td>4.3 ± 0.6(^c)</td>
</tr>
<tr>
<td>Follicle size(^3) (mm)</td>
<td>17.2 ± 1.1</td>
<td>16.5 ± 1.6</td>
<td>16.2 ± 1.8</td>
</tr>
</tbody>
</table>

\(^1\)\(^{a,b}\) \((P = .006)\)

\(^2\)\(^{a,b,c}\) \((P = .0001)\)
Concentrations of Estradiol-17β

- **CON**
- **TRT-EST**
- **TRT-NOEST**

Estrogen (pg/ml)

Hours from maximum LH

(*P=.04)
Summary

- Preovulatory LH surge and LH pulsatility are suppressed during acute clinical mastitis.

- Estradiol remained at basal levels in cows following experimentally induced clinical (acute) mastitis.
Mastitis

⇓⇓ ⇓⇓
Reproduction

PGF

Body Temp

Immune Response

(-)

Embryo Development

Oocyte Maturation

Ovulation

Pregnancy Rates

LH & FSH

Cortisol

(-)

Follicular Development

Hypothalamo-Pituitary-Ovarian Axis

Mastitis ↓ Reproduction

PGF$_{2\alpha}$

Feed Intake

Altered Metabolites

(-)

Hormonal Alterations

Follicular Development

Pregnancy Rates

Uterine-Ovarian Axis

Nutritional Interaction

Pregnancy Rates

Luteal Length
Hypothalamo-pituitary-ovarian axis

Hypothalamus → GnRH

↓

Pituitary → LH

↓

Ovary

+ Estrogen

Ovulation
Hypothalamo-pituitary-ovarian axis

Hypothalamus → + → GnRH

Pituitary

LH

Ovary

Estrogen

Expression of estrus

Mastitis (Cortisol)

“-”

Ovulation
Conclusions

- Clinical mastitis resulting from *S. uberis* bacterial challenge resulted in
  - Inflammatory response
  - Increased body temperature
  - Increased cortisol
  - Increased PGF$_{2\alpha}$
  - Decreased milk production
  - Elevations in BUN & NEFA
  - Decreased LH released following GnRH
  - Decreased LH pulsatility
  - Decreased E2 Production
Conclusions

- Alterations in hypothalamo-pituitary-ovarian axis play a role in decreased estrous expression and ovulation.

- Clinical mastitis prior to ovulation may result in increased days to first service, days open and services required per conception by alterations in endocrine function.