Matrix Metalloproteinases as Plasma Indicators of Bovine Cystic Ovarian Disease

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Introduction

- One of the main causes of infertility in dairy cattle
- Endocrine imbalance within the hypothalamus-pituitary-ovarian axis
- Prevents the mature follicle from ovulating
Objective

To determine if plasma concentrations of matrix metalloproteinases (MMP) and/or their tissue inhibitors (TIMP) differed between healthy cows and cows with COD.
Day 0: Estradiol from follicle → Gonadotropin releasing hormone (GnRH) released → Luteinizing hormone (LH) surge → Ovulation → Oocyte released
Estrous Cycle

- Day 1-5: Follicle luteinizes → corpus luteum (CL) formation
- Day 9-10: Maximum Progesterone production from CL
Day 16-18

- Pregnant: Embryo blocks prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) → CL of pregnancy produces progesterone → pregnancy maintained
- Open: PGF$_{2\alpha}$ from uterus → CL regresses → decrease in progesterone → LH & follicle stimulating hormone (FSH) increase → new wave of follicles
Follicular Development

- Recruitment, selection, dominance
- Many follicles developing at a time
- One becomes dominant
- Remaining undergo atresia & regress
- Dominant continues to grow and becomes mature follicle containing the oocyte
Estrous Cycle and Associated Hormones
Cystic Ovarian Disease

- **Follicular**
  - Single or multiple
  - Thin walled
  - Low progesterone
  - 70% of COD cases

- **Luteal**
  - Usually single
  - Thick wall
  - High progesterone
Follicular Cysts

- Follicle that has failed to ovulate
- Diameter greater than 2.5 cm
- Persist for >10 days
- Affects up to 30% of high producing dairy cows
- Cause currently unknown
Predisposition

- Seasonal Changes
- Increasing age
- Parity
- Body Condition

- Nutrition
- Infectious Diseases
- Herd Management
- Genetics
Economic Impact

- Each case $150-180 to treat ($3.00/cow/day)
- $1.875 million per year for Oregon producers
- $139.5 million nationally
Economic Impact

- ↑ Postpartum interval
- ↑ Breeding costs
- ↑ Calving interval
- ↑ Medical cost
- ↑ Culling rates
Factrel® / Cystorelin®
- Gonadotropin releasing hormone (GnRH)
- Induces LH from pituitary
- Luteinizes cyst
• **Lutalyse®**
  - PGF$_{2\alpha}$

• Causes regression of CL
  - ↓Progesterone

• Allows estrus & ovulation

• Cows bred on next estrus
Breakdown of extracellular matrix (ECM) critical for ovulation

Breakdown of ECM dependent on extracellular matrix degrading proteins
- Plasminogen activator system (PA)
- Matrix metalloproteinase system (MMP)
Plasminogen Activator

- Converts plasminogen into plasmin
- Plasmin degrades ECM & activates pro-MMP
- Tissue-type plasminogen activator (tPA)
- Urokinase-type plasminogen activator (uPA)
OSU PA Study (McNeel & Menino 2011)

- No significant differences in tPA or plasminogen activator inhibitor-1 (PAI-1) plasma concentrations or ratios
- Gene expression of tPA and PAI-1 at the follicular level were not different
- More uPA expression in preovulatory vs. cystic follicles
- Less uPA receptor (uPAR) expression in preovulatory vs. cystic follicles
PAI-1 to uPA ratio greater in cysts

Gene expression altered in follicular cysts in favor of protease inhibition
Serine protease inhibitor E1 (SERPINE1)
4 basepair insertion/deletion in 27 of 78 cows
- Jerseys 56.4% deletion rate
- Holsteins 12.8% deletion rate
Plasma PAI-1 concentration not affected based on insertion/deletion

OSU PA Study (McNeel & Menino 2011)
Matrix Metalloproteinases

- Proteolytic enzymes
- Zinc-dependent proteinases
- Important for many cell behaviors
- Reorganization of ECM
- Participate in ovulation
MMP Activity in COD

  - Contradictory
  - Lower inhibin
  - Regulates FSH
Tissue inhibitor of matrix metalloproteinase (TIMP)

- High TIMP ratios associated with ECM buildup
- TIMP-1 constitutively expressed
- TIMP-2 directly inhibits MMP-2
- TIMP-2 is also required for MMP-2 activation
Polycystic Ovarian Syndrome

- Similar to COD
- Infertility in women (10-20%)
- High number of antral follicles
- Often associated with hyperandrogenism, insulin resistance and potentially type-2 diabetes
Polycystic Ovarian Syndrome

- Single-nucleotide polymorphism (SNP) in the promoter region of SERPINE1 gene
- Plasma PAI-1 25% higher in women with PCOS
- Circulating serum concentrations MMP-2 and -9 and TIMP-1 significantly higher (Lewandowski et al. 2006)
- Follicular fluid MMP-2 and -9 1.6 and 1.7 fold higher, respectively (Shalev et al. 2001)
Hypothesis

- Plasma MMP-2 and -9 will be lower and TIMP-1 and-2 higher in cows with follicular cysts compared to non-cystic cows
  - Imbalance favoring TIMP, which makes the cow proteolytically disabled
Blood Plasma Analysis

daviddarling.info
Mistrymedical.com
Hce-uk.com
Animals

- 65 lactating cows
  - 4 at OSU Dairy Center in Corvallis, Oregon
  - 61 at Konyn Dairy in Coburg, Oregon
- Konyn Dairy total 1,500 milking cows
- Free choice water & total mixed ration
Blood Collection

- Cystic
  - Diagnosed via rectal palpation
- Non-cystic (control)
  - No cyst history
  - Diagnosed as in-heat
    - Stand for mounting
    - Increased steps
    - Decreased milking
Blood Collection

- Tail venipuncture
Plasma Collection

- Centrifuge
  - Plasma on top
  - Red blood cells on bottom
- Package
  - Cryotubes
- Freeze

Plasma:
- Water, proteins, nutrients, hormones, etc.

Buffy coat:
- White blood cells, platelets

Hematocrit:
- Red blood cells

Normal Blood:
Elisa

- Enzyme-linked immunosorbent assay
- Read plate according to ELISA manufacturers instructions
- More color = higher concentration
Calculations

- Standard curve equation from Excel
- Insert OD readings $\rightarrow$ calculate concentrations
Results
MMP-2 Concentrations

Cystic mean = 228.8 pg/ml

Non-cystic mean = 311.5 pg/ml

Cyst cows 36% higher

P=0.33
MMP-9 Concentrations

Cystic mean = 89.5 pg/ml

Non-cystic mean = 95.4 pg/ml

P=0.76
TIMP-1 Concentrations

Cystic mean = 4.71 ng/ml

Non-cystic mean = 4.96 ng/ml

P=0.86
TIMP-2 Concentrations

Cystic mean = 39.7 ng/ml
Non-cystic mean = 36.9 ng/ml

P=0.15
Tied directly to MMP-2 regulation
MMP-2 vs. No. of Cyst

Trends down, except 3-cyst group

P=0.79
MMP-9 vs. No. of Cyst

P=0.98

3-cyst group is the lowest
TIMP-1 vs. No. of Cyst

P=0.90

Trends down as # of cyst increases
TIMP-2 vs. No. of Cyst

P = 0.46

3-cyst had highest levels
TIMP-1: MMP-9 Ratio

Cystic mean = 421.0
Non-cystic mean = 224.1
P<0.10

Cystic cows have a ratio favoring TIMP
TIMP-2: MMP-2 Ratio

Cystic mean = 3510.6

Non-cystic mean = 958.9

P < 0.04

Cystic cows have a ratio favoring TIMP
One cyst group TIMP-1:MMP-9 ratio 2-fold greater than non-cystic (P<0.05)

One cyst group TIMP-2:MMP-2 ratio 4-fold greater than non-cystic (P<0.05)
TIMP-1:MMP-9 & TIMP-2:MMP-2 molar ratio correlations with age and parity were negative for cystic cows.

- TIMP-1:MMP-9 approached statistical significance (P=0.06 for age & P=0.12 for parity).

- No significant correlations in molar ratios for non-cystic cows.
Discussion - MMP

No significance differences in plasma concentration
- MMP higher in non-cystic cows
- MMP-2 plasma concentration 36% higher in non-cystic

With larger sample sizes significant differences may have been realized

Results differ from some studies
TIMP-1 similar in both groups
  - Constitutively expressed
TIMP-2 elevated in cystic and approached significance
  - Larger sample may have detected significant differences
TIMP-2 tightly tied to MMP-2 regulation
MMP-9 regulated by multiple TIMP
Conflicts with some other studies
TIMP-1: MMP-9 ratio favored TIMP 100 fold
TIMP-2: MMP-2 ratio favored TIMP 1000 fold
Both ratios favored protease inhibition in cystic cows
  - Reduced proteolysis in cows with COD
Suggests plausible explanation for the follicular cyst pathology
Discussion – Age & Parity

- TIMP-1: MMP-2 molar ratio approached significance for cystic cows
  - As age & parity increased, ratio decreased
- TIMP-2: MMP-2 ratio was nonsignificant
Conclusions

- Imbalance in ECM degrading proteinase systems could play a role in COD
- If difference in molar ratios translates to the ovarian level the impaired proteolysis may predicate development of the follicular cyst pathology
- TIMP-2: MMP-2 molar ratio as marker for heifers more likely to develop COD
- Undetermined
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