



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



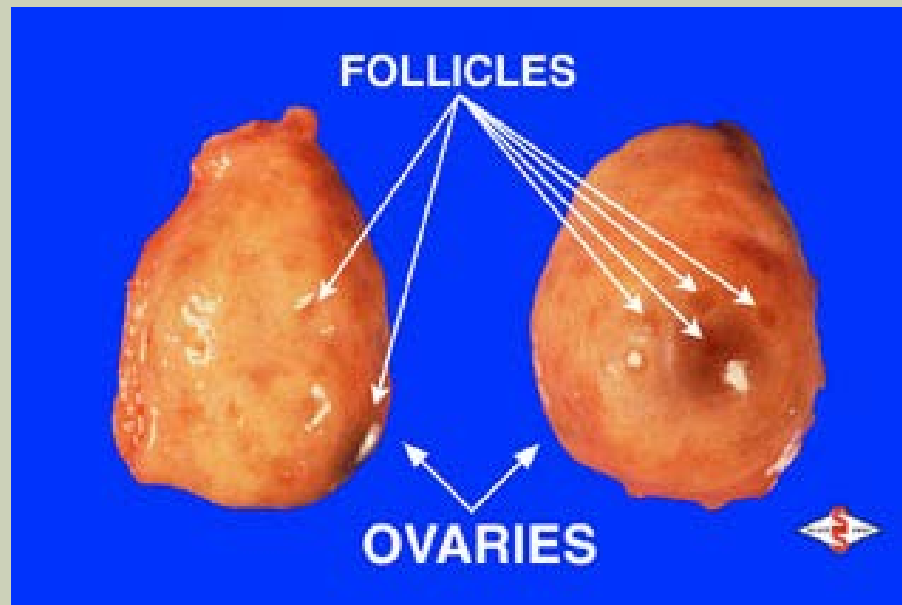
Objective

- To determine if plasma concentrations of matrix metalloproteinases (MMP) and/or their tissue inhibitors (TIMP) differed between healthy cows and cows with COD



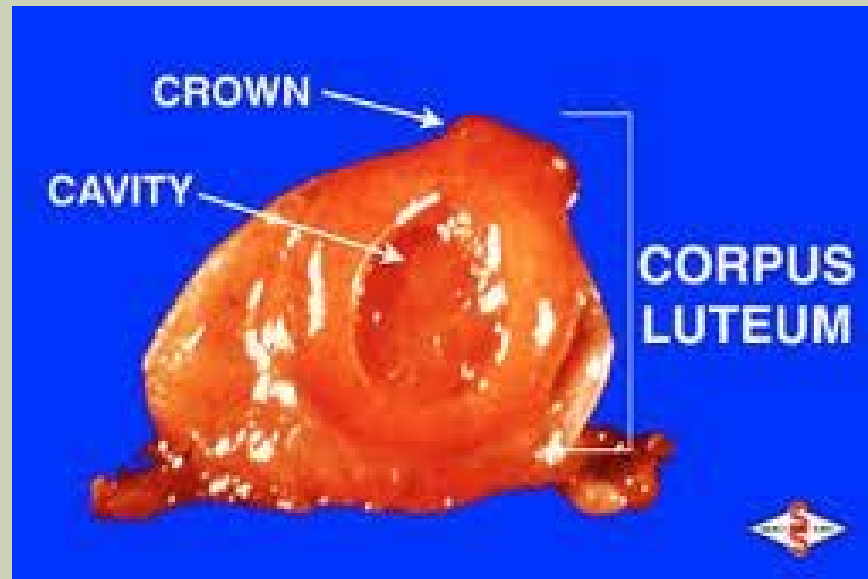
Estrous Cycle

- 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



Estrous Cycle

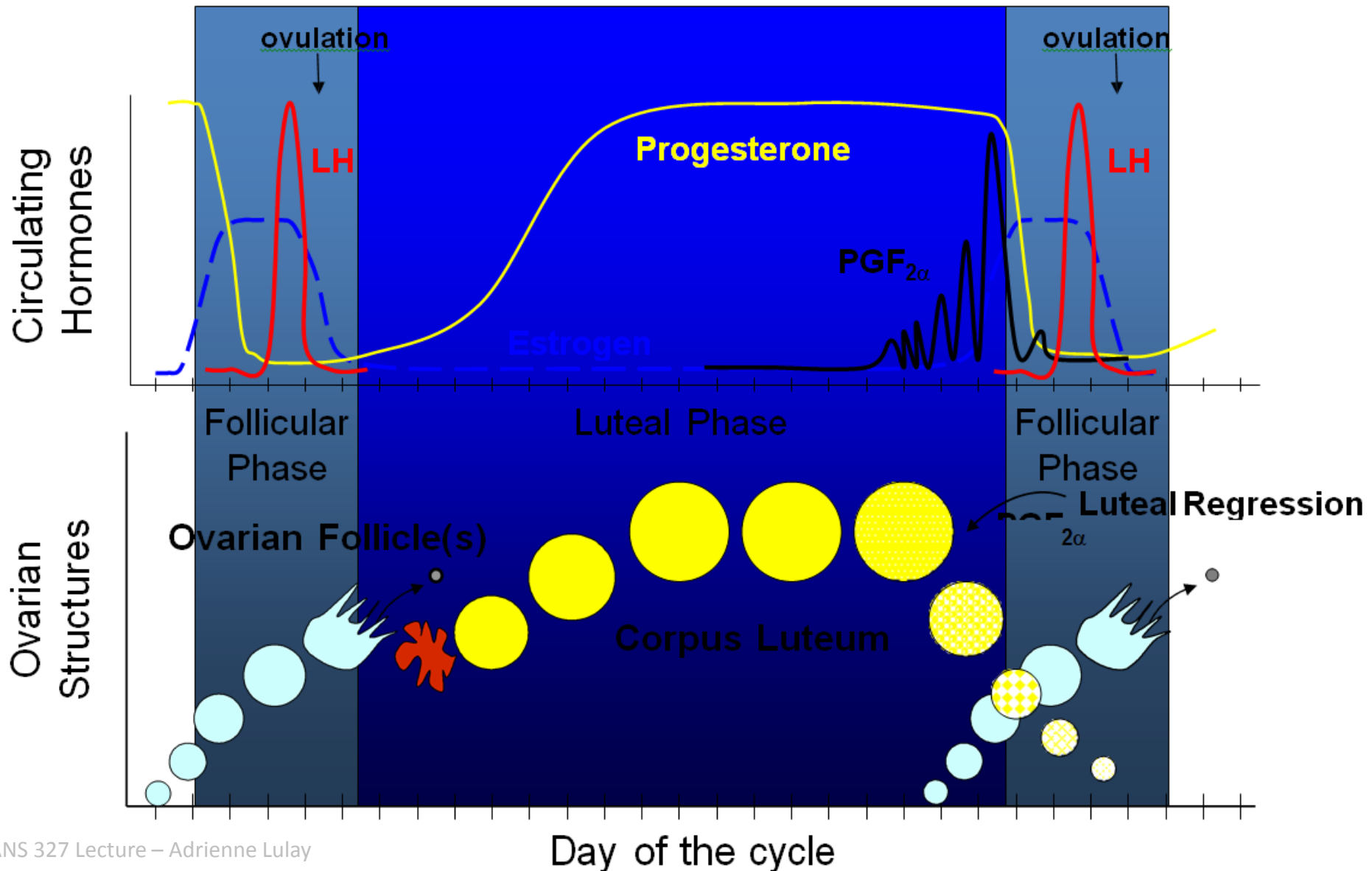
■ Day 16-18

- 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

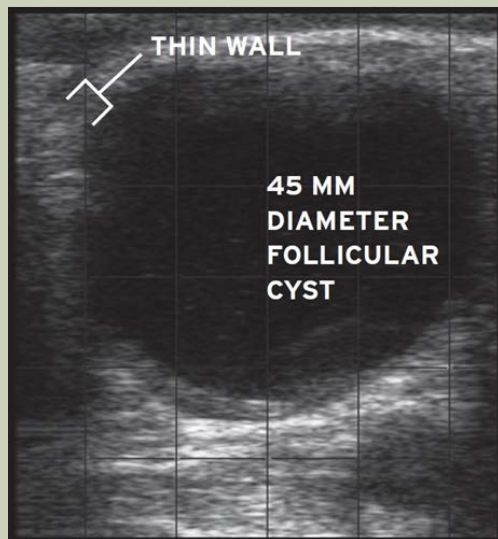
Estrous Cycle and Associated Hormones



Cystic Ovarian Disease

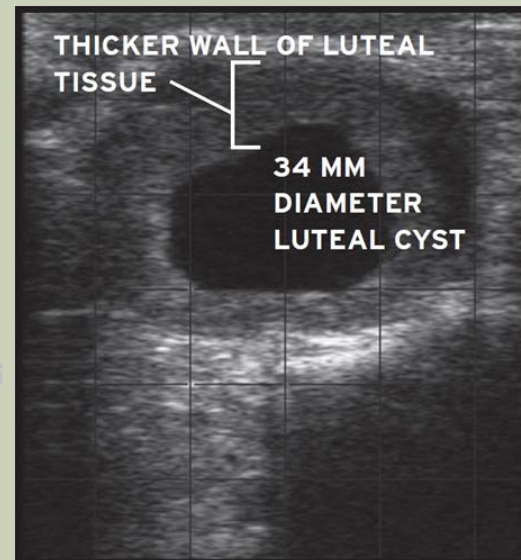
■ Follicular

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



■ Luteal

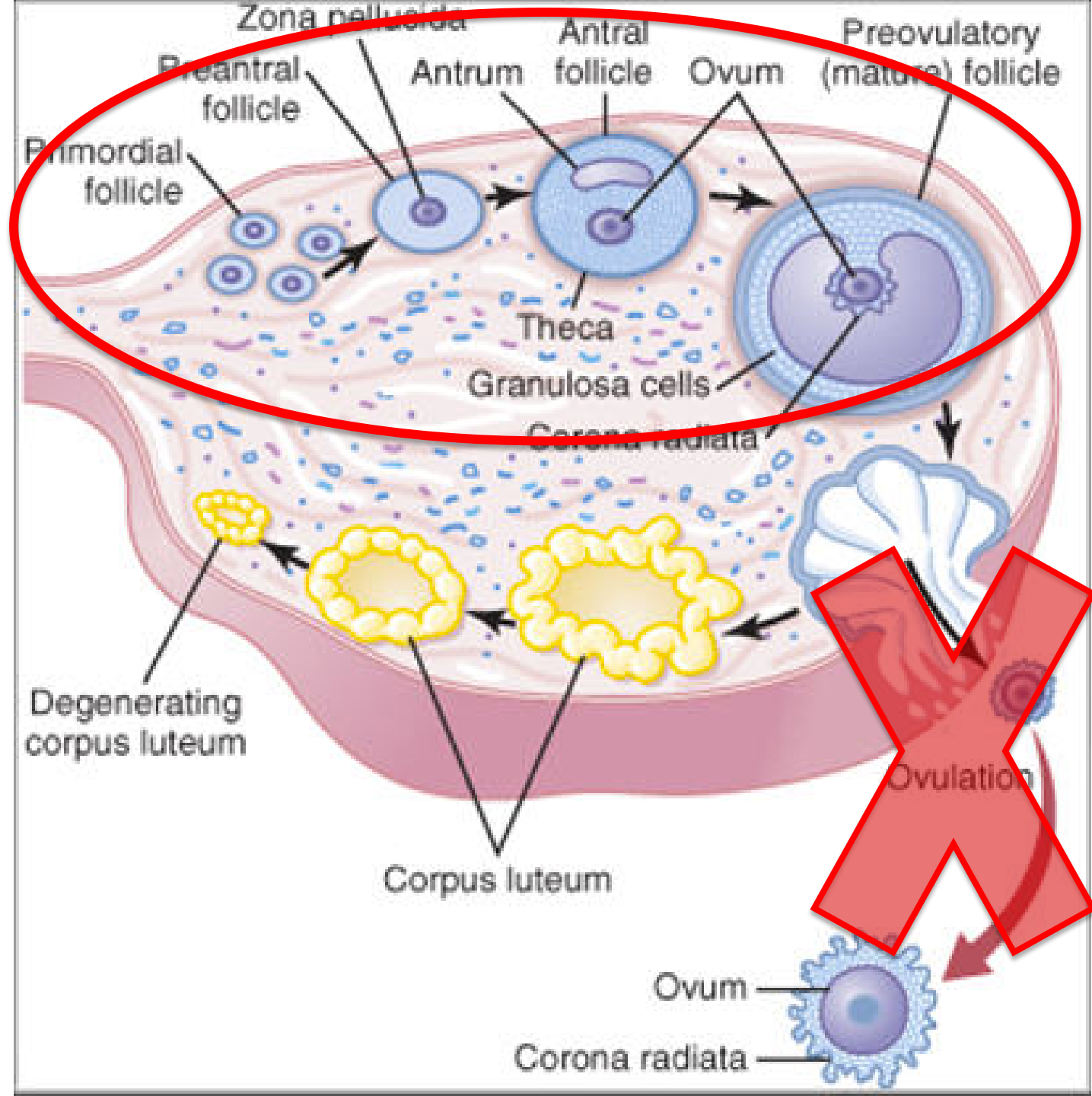
- Usually single
- Thick wall
- High progesterone

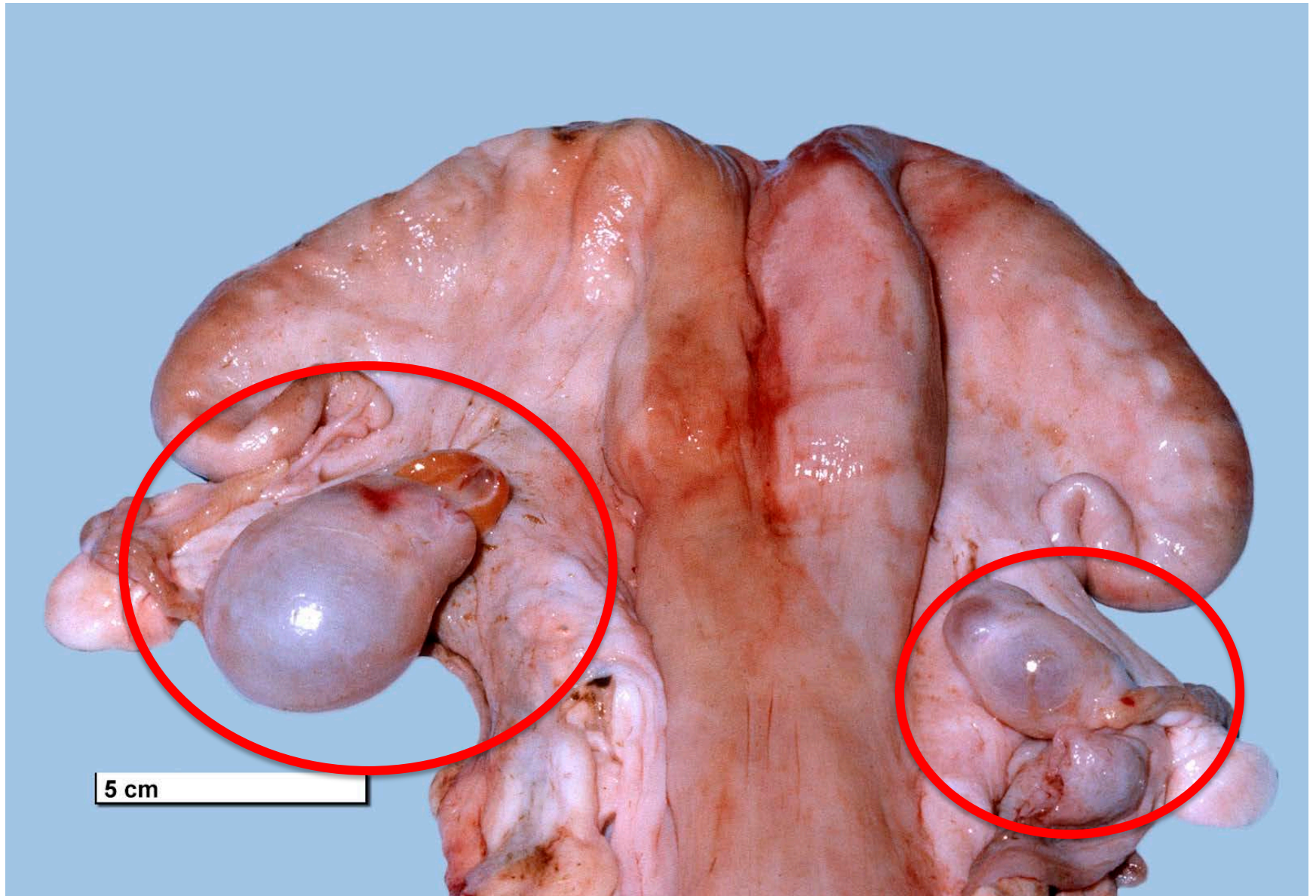


Follicular Cysts

- 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 mil



Economic Impact

- ↑ Postpartum interval
- ↑ Breeding costs
- ↑ Calving interval
- ↑ Medical cost
- ↑ Culling rates



Treatment

- Factrel[®] / Cystorelin[®]
 - Gonadotropin releasing hormone (GnRH)
- Induces LH from pituitary
- Luteinizes cyst



Treatment

- Lutalyse®
 - PGF_{2α}
- Causes regression of CL
 - ↓ Progesterone
 - Allows estrus & ovulation
- Cows bred on next estrus



ECM Degradation

- 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

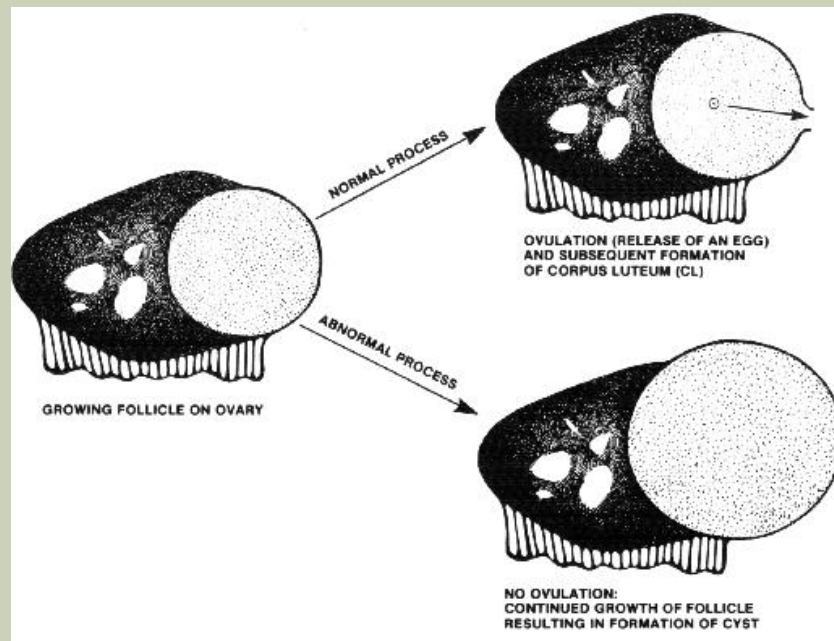
- Converts plasminogen into plasmin
- Plasmin degrades ECM & activates pro-MMP
- Tissue-type plasminogen activator (tPA)
- Urokinase-type plasminogen activator (uPA)

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

OSU PA Study (McNeel & Menino 2011)

- PAI-1 to uPA ratio greater in cysts
- Gene expression altered in follicular cysts in favor of protease inhibition

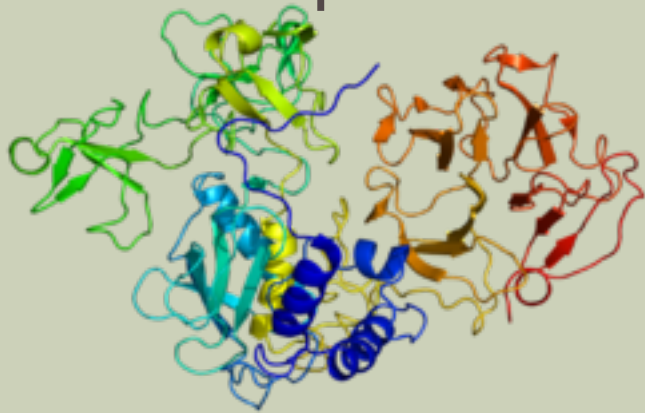


OSU PA Study (McNeel & Menino 2011)

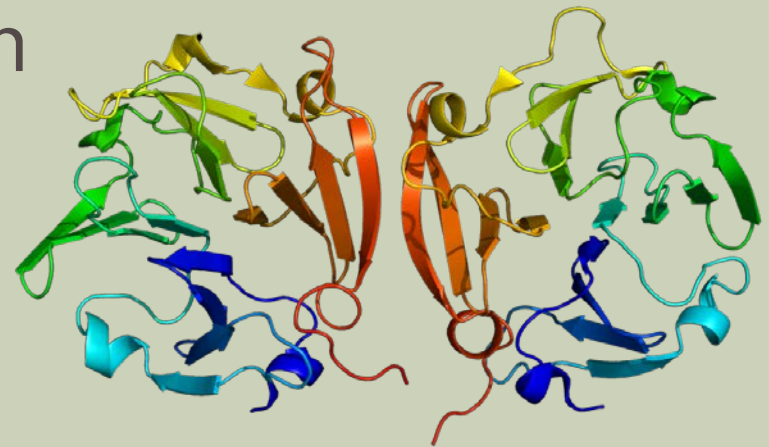
- Serine protease inhibitor E1 (SERPINE1)
- 4 basepair insertion/deletion in 27 of 78 COWS
 - Jerseys 56.4% deletion rate
 - Holsteins 12.8% deletion rate
- Plasma PAI-1 concentration not affected based on insertion/deletion

Matrix Metalloproteinases

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



http://commons.wikimedia.org/wiki/File:Protein_MMP2_PDB_1ck7.png



http://en.wikipedia.org/wiki/Matrix_metalloproteinase#/media/File:Protein_MMP9_PDB_1itv.png

MMP Activity in COD

- Higher proMMP-2 & -9 activity in presumably cystic follicles (Imai *et al.* 2003)
 - Contradictory
- Lower inhibin
 - Regulates FSH



TIMP

- 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

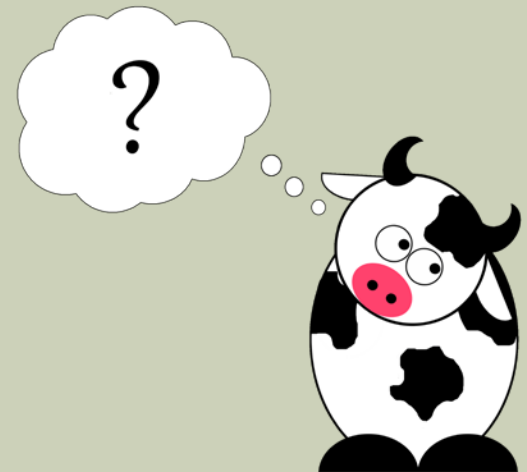
- Similar to COD
- Infertility in women (10-20%)
- High number of antral follicles
- Often associated with hyperandrogenism, insulin resistance and potentially type-2 diabetes

Polycystic Ovarian Syndrome

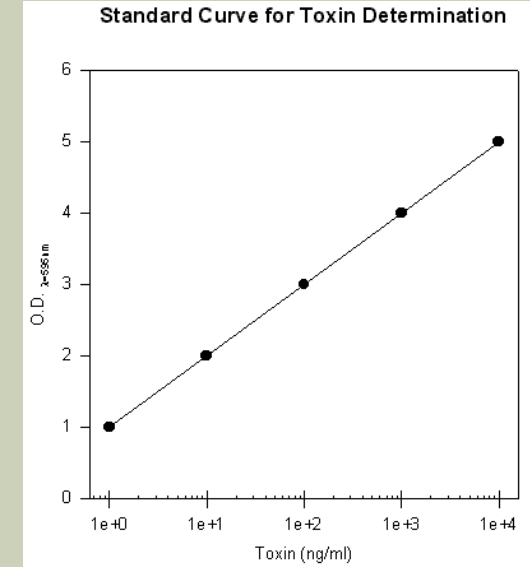
- Single-nucleotide polymorphism (SNP) in the promoter region of SERPINE1 gene
- Plasma PAI-1 25% higher in women with PCOS
- Circulating serum concentrations MMP-2 and -9 and TIMP-1 significantly higher (Lewandowski *et al.* 2006)
- Follicular fluid MMP-2 and -9 1.6 and 1.7 fold higher, respectively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

Hypothesis

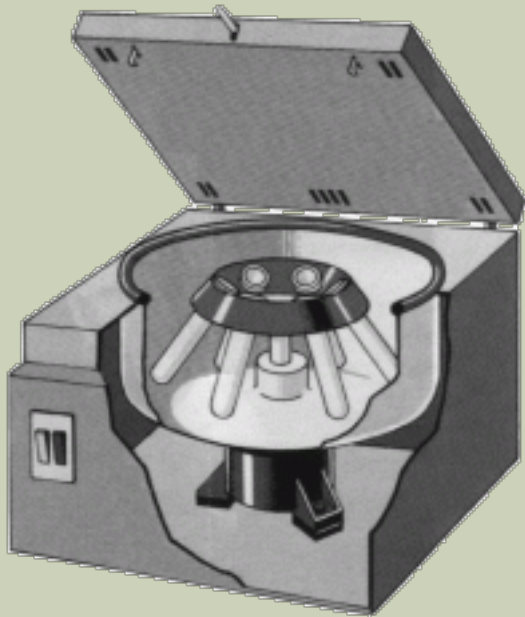
- Plasma MMP-2 and -9 will be lower and TIMP-1 and -2 higher in cows with follicular cysts compared to non-cystic COWS
 - Imbalance favoring TIMP, which makes the cow proteolytically disabled



Materials & Methods



Blood Plasma Analysis



daviddarling.info



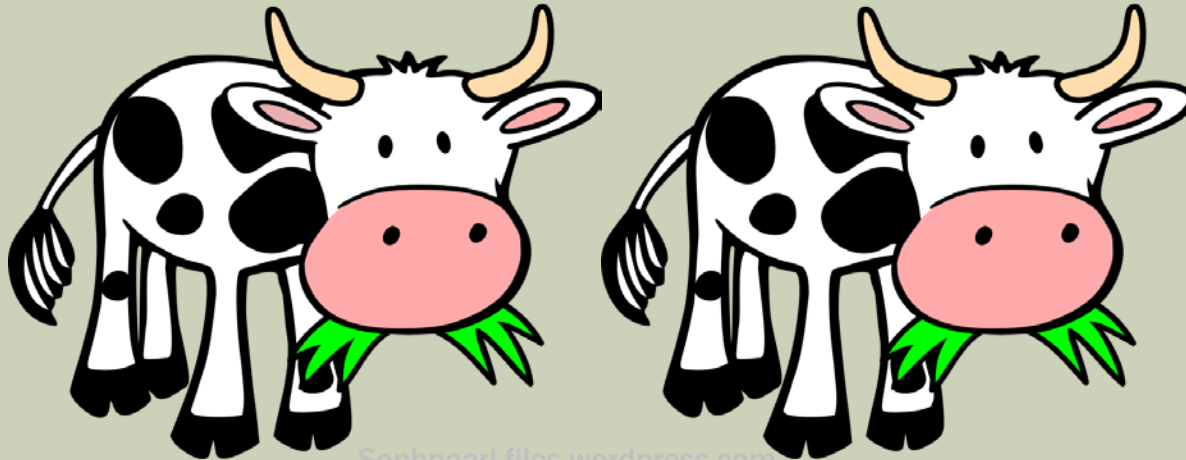
Mistrymedical.com



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



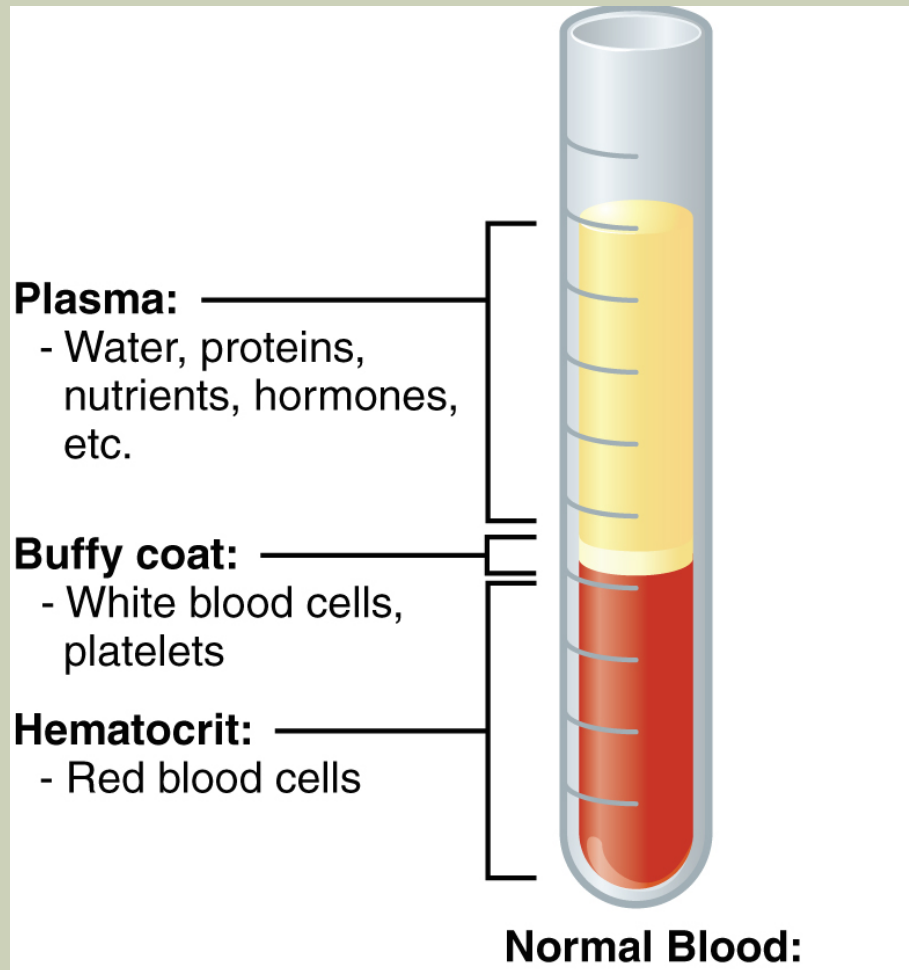
Blood Collection

■ Tail venipuncture



Plasma Collection

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



ELISA

- Enzyme-linked immunosorbent assay



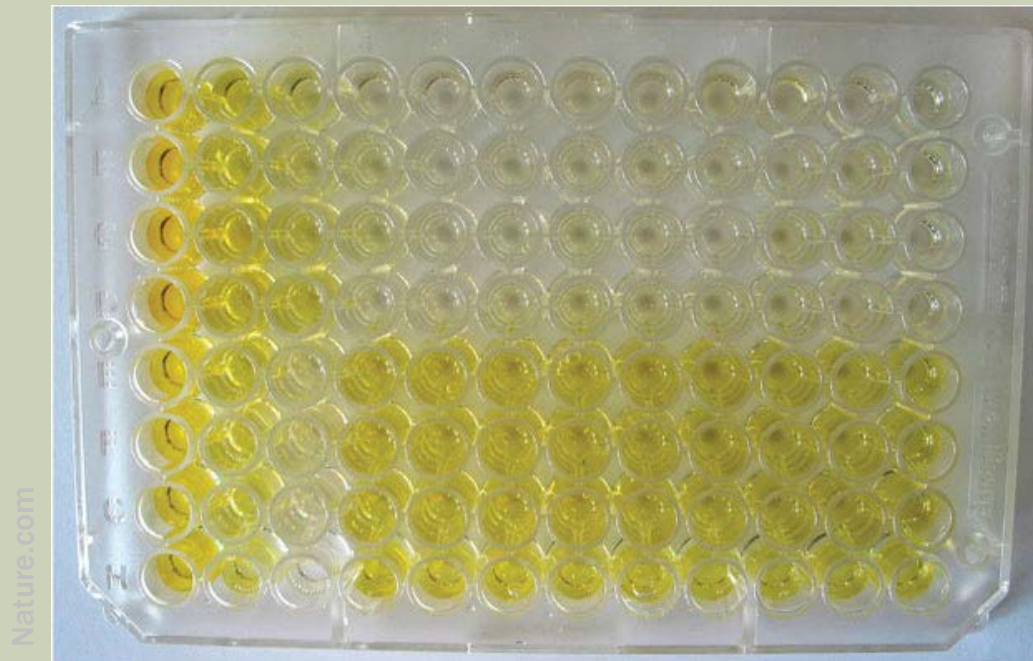
Plate Reading

- Read plate according to ELISA manufacturers instructions
- More color = higher concentration



Calculations

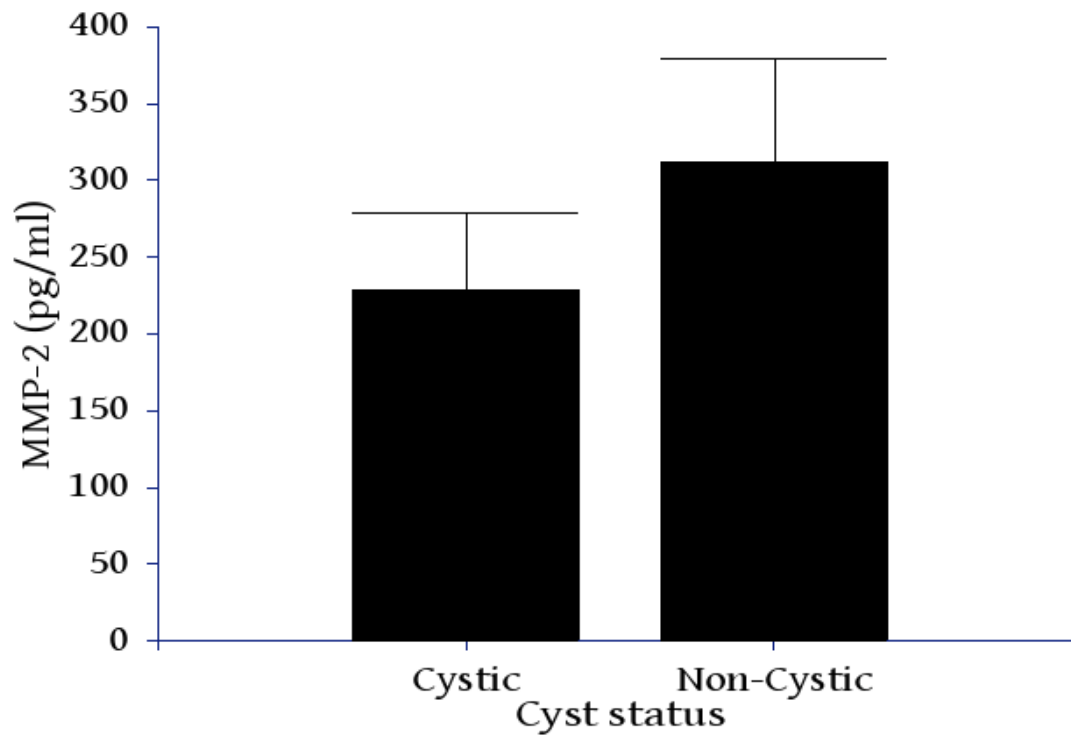
- Standard curve equation from Excel
- Insert OD readings → calculate concentrations



Results



MMP-2 Concentrations



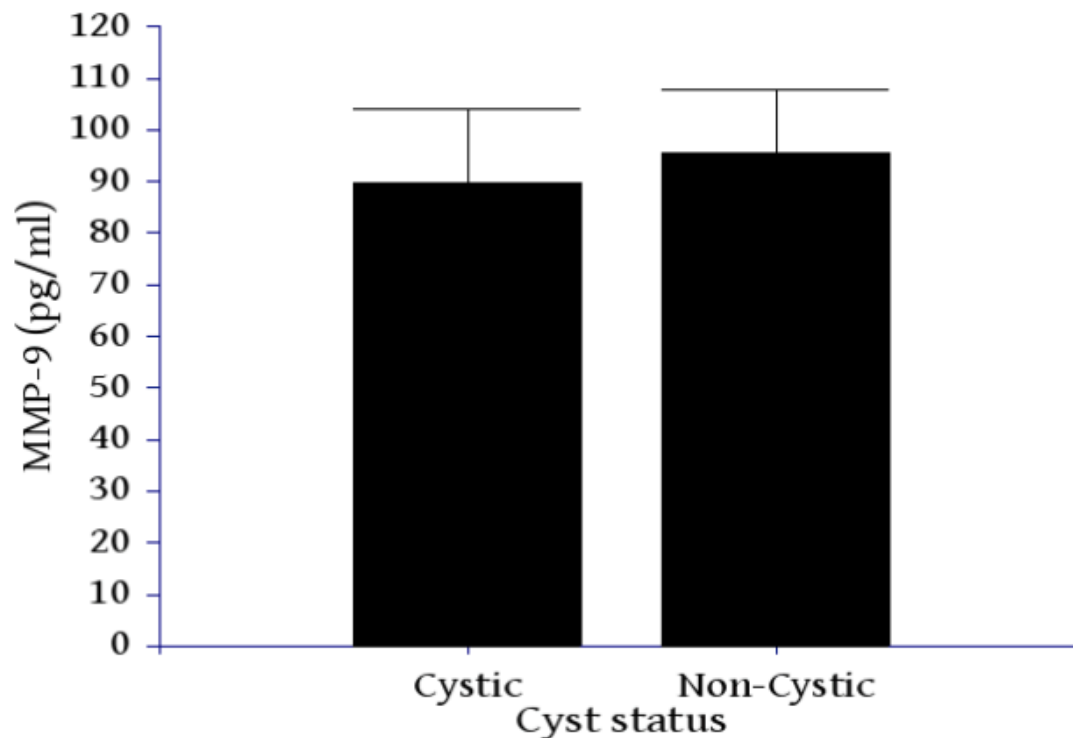
**Cystic mean =
228.8 pg/ml**

**Non-cystic mean
= 311.5 pg/ml**

**Cyst cows 36%
higher**

P=0.33

MMP-9 Concentrations

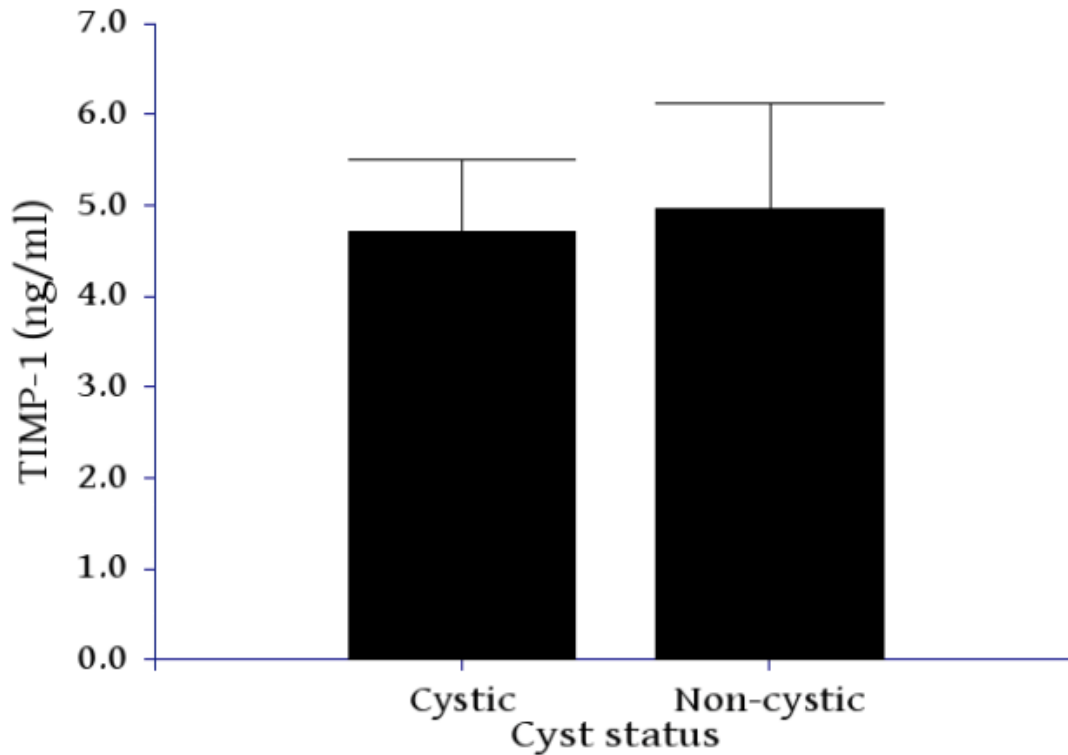


Cystic mean =
89.5 pg/ml

Non-cystic mean
= 95.4 pg/ml

P=0.76

TIMP-1 Concentrations

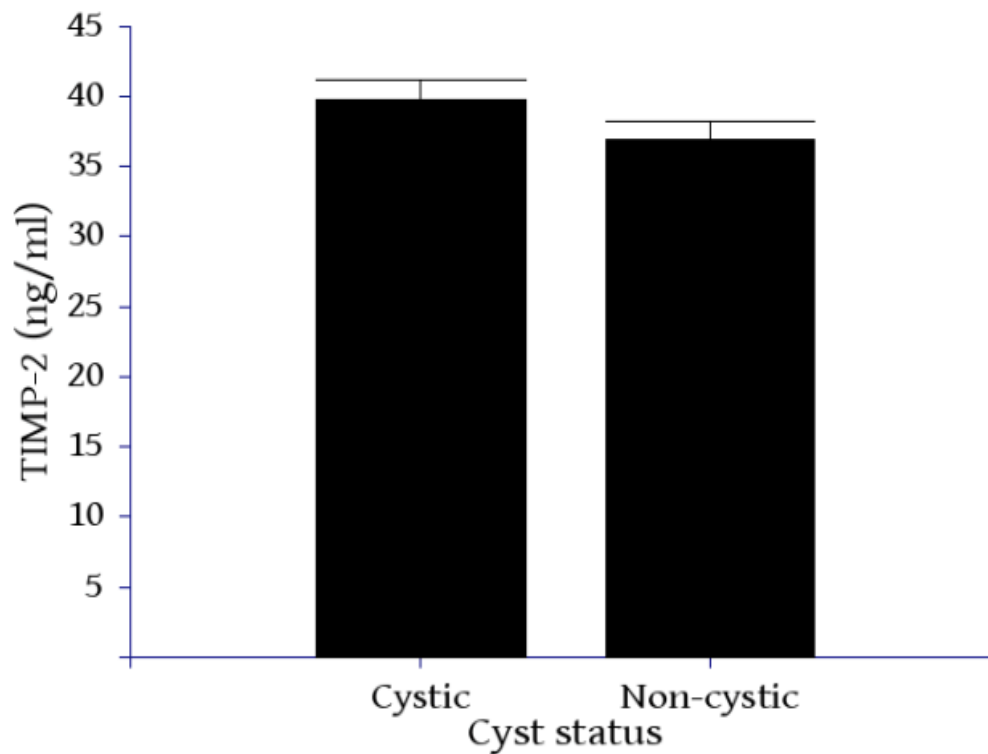


**Cystic mean =
4.71 ng/ml**

**Non-cystic mean
= 4.96 ng/ml**

P=0.86

TIMP-2 Concentrations



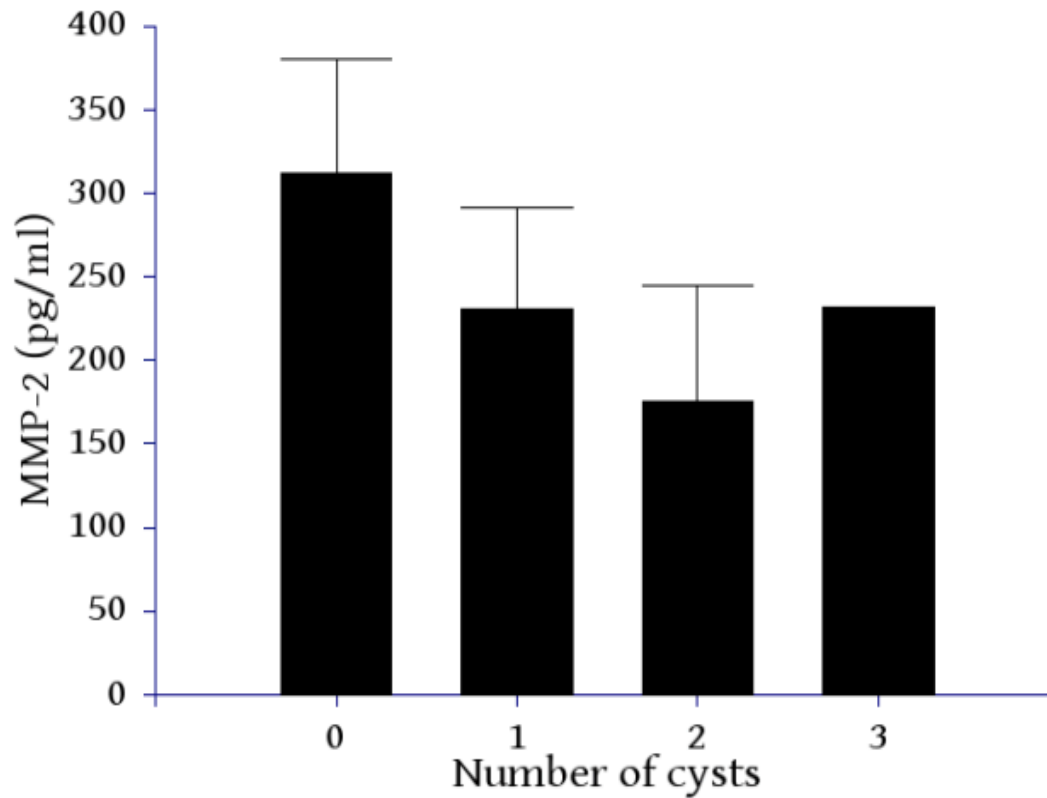
**Cystic mean =
39.7 ng/ml**

**Non-cystic mean
= 36.9 ng/ml**

P=0.15

**Tied directly to
MMP-2
regulation**

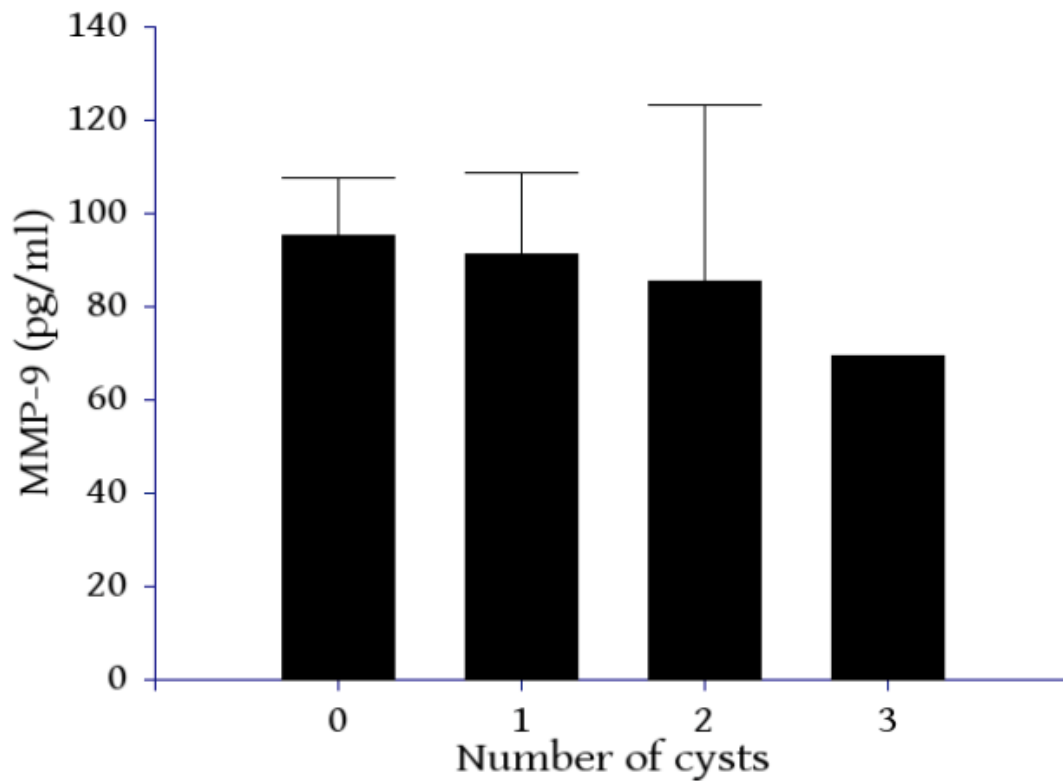
MMP-2 vs. No. of Cyst



P=0.79

**Trends down,
except 3-cyst
group**

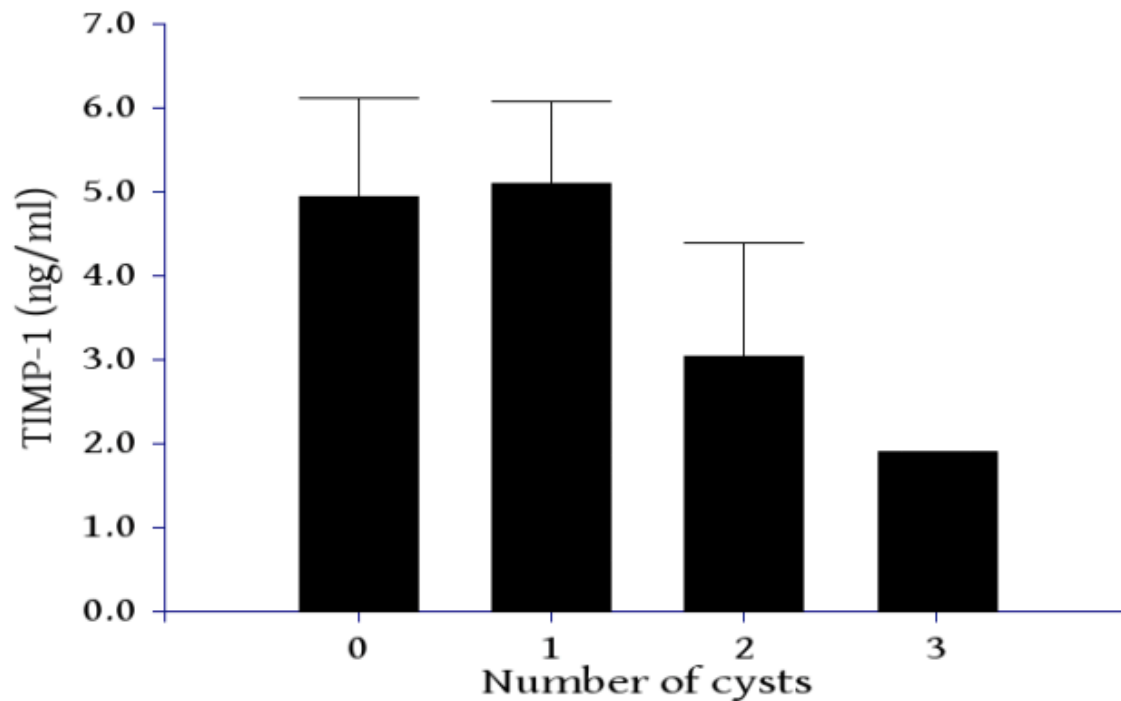
MMP-9 vs. No. of Cyst



P=0.98

**3-cyst group is
the lowest**

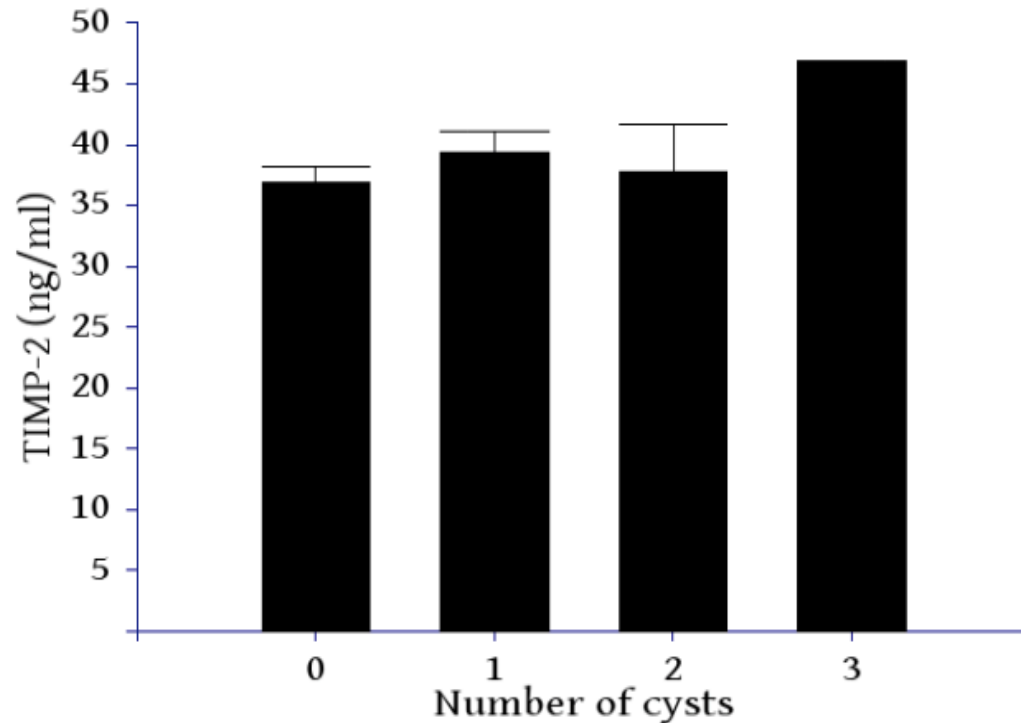
TIMP-1 vs. No. of Cyst



P=0.90

**Trends down as
of cyst
increases**

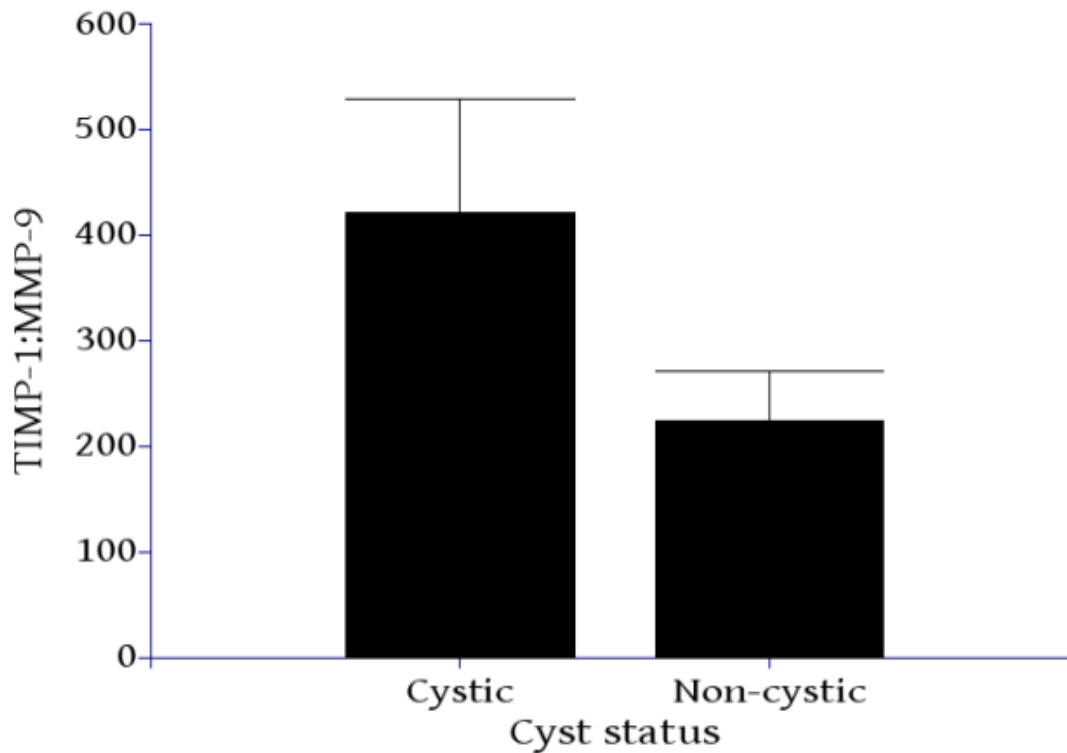
TIMP-2 vs. No. of Cyst



P=0.46

**3-cyst had
highest levels**

TIMP-1:MMP-9 Ratio



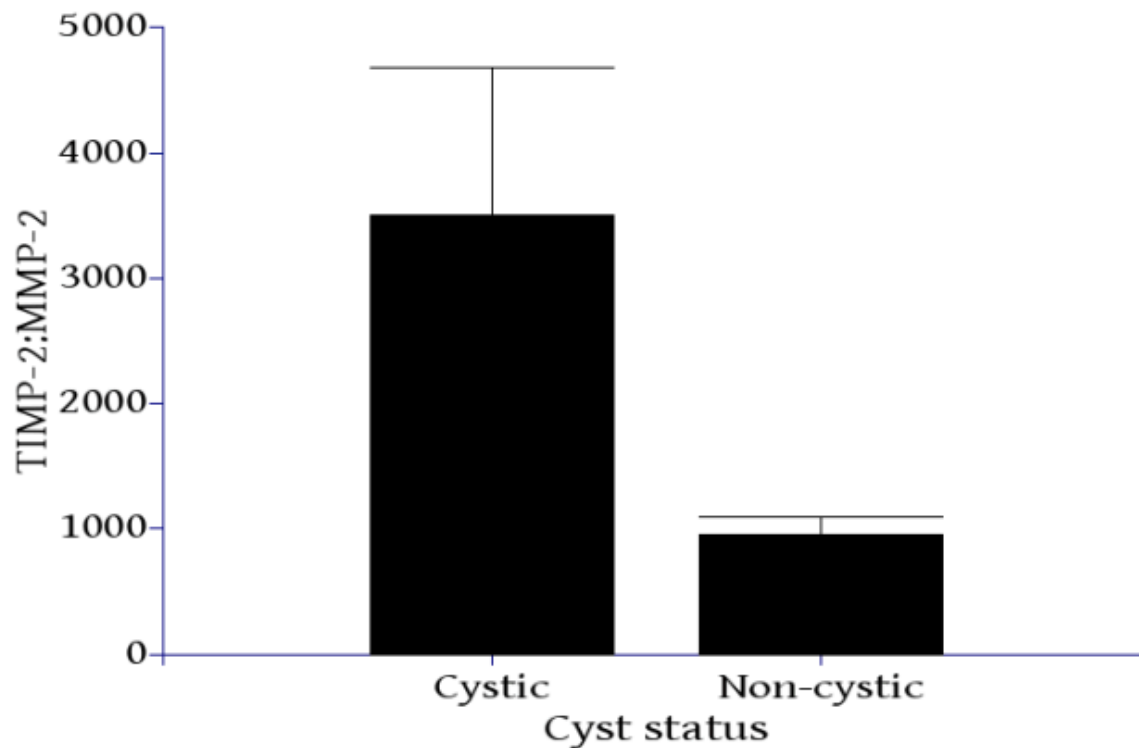
**Cystic mean =
421.0**

**Non-cystic mean
= 224.1**

P<0.10

**Cystic cows have
a ratio favoring
TIMP**

TIMP-2:MMP-2 Ratio



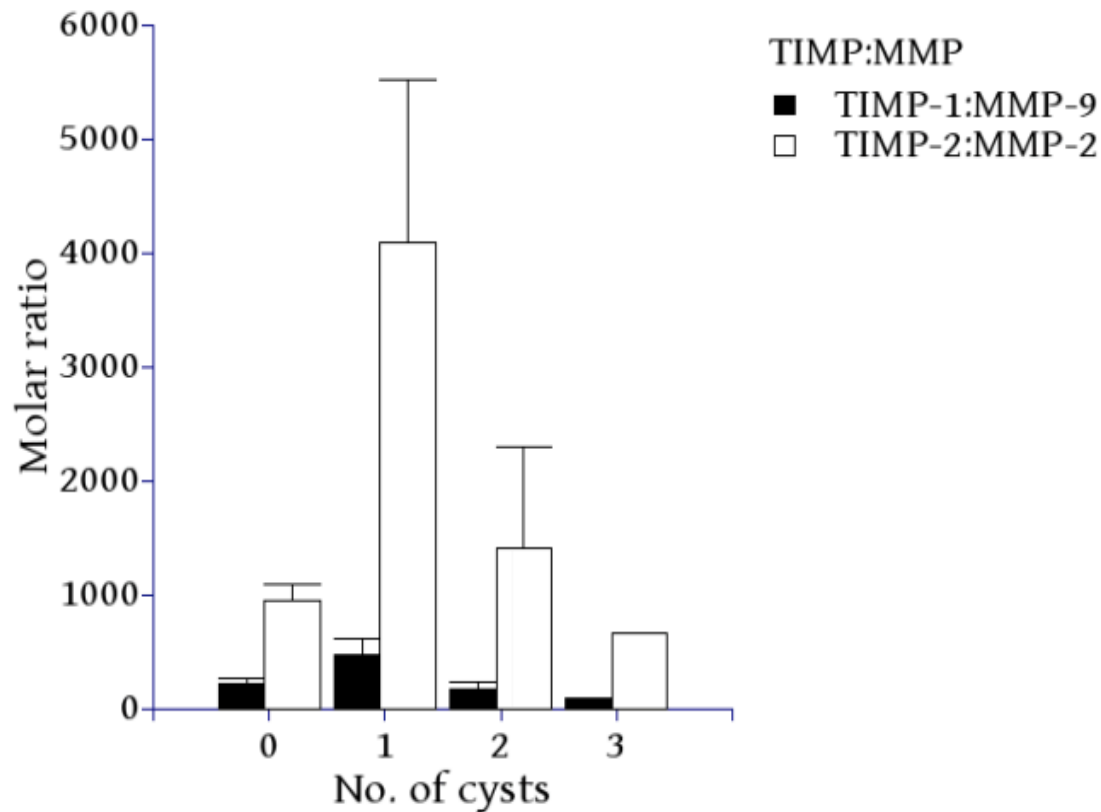
**Cystic mean =
3510.6**

**Non-cystic mean
= 958.9**

P<0.04

**Cystic cows have
a ratio favoring
TIMP**

Molar Ratio vs. No. of Cyst



**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
($P < 0.05$)**

Age & Parity Analysis

- 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

Discussion - TIMP

- 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

Discussion – Molar Ratio

- 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

Acknowledgements

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