EPIGENETIC REGULATION OF MILK PRODUCTION IN DAIRY COWS

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LACTATION BIOLOGY TEAM
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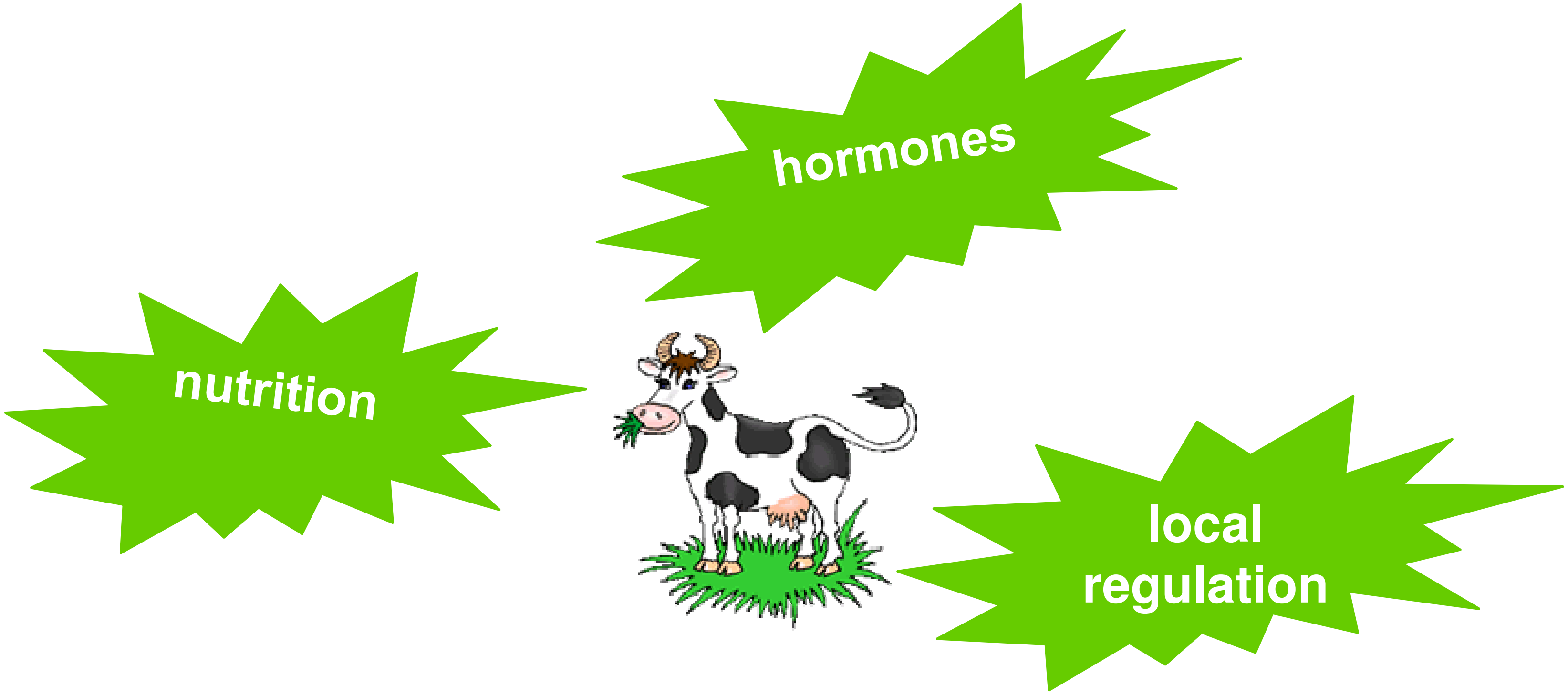
OVERVIEW



hormones

nutrition

local regulation

Milk production is a function of mammary cell number and activity

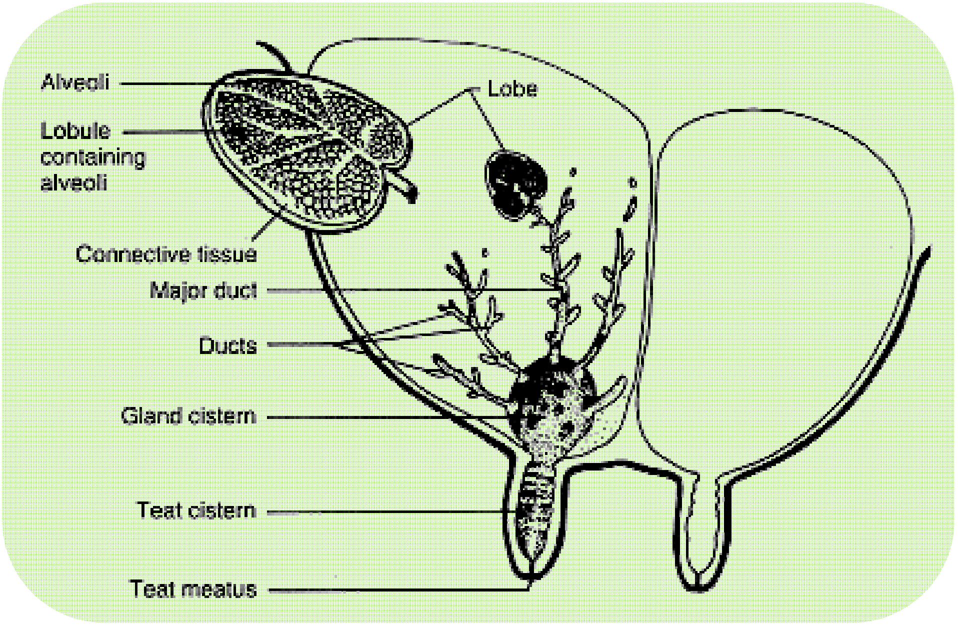
OVERVIEW

Primary goal

To understand how the mammary gland is able to respond to environmental cues for enhanced milk production

Overall hypothesis

Milk production can be influenced by environmental factors through cell signalling and epigenetic mechanisms



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OUTLINE OF TODAY'S TALK

- Introduction - Epigenetic mechanisms
- Transgenerational epigenetic inheritance
- Dynamic epigenetic mechanisms

EPIGENETICS

- Chemical modifications of the DNA/chromatin which cause changes in phenotype (appearance) or gene expression
- Influenced by environmental factors
- Inherited

→ **Can we manipulate the environment for improved life-time consequences of the dam and the off-spring**

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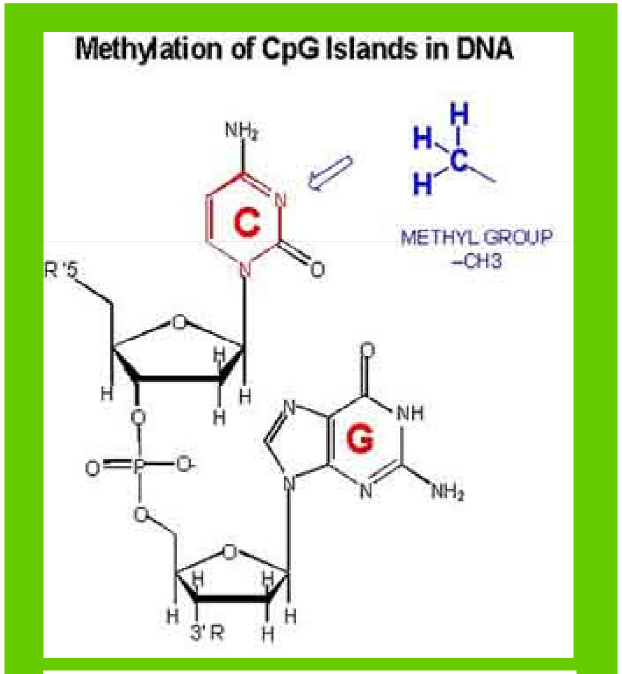
EPIGENETICS

Gene expression altered by:

- DNA Methylation (CpG) sites
- Histone modification and chromatin remodelling



5'–CpG–3'

3'–GpC–5'

DNA helix with methyl groups (red)
(picture by Dr C Cooney)

PART TWO

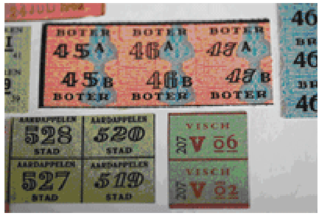
- Introduction - Epigenetic mechanisms
- **Transgenerational epigenetic inheritance**
- Dynamic epigenetic mechanisms

EPIDEMIOLOGICAL EVIDENCE FOR TRANSGENERATIONAL EPIGENETIC INHERITANCE IN HUMANS

- The Dutch Famine of 1944 - *Lumey, 1992 Paediatr Perinat Epidemiol 6:240*
- Children of pregnant women exposed to famine were
  - smaller than average
  - more susceptible to health problems
    (eg diabetes, obesity, cardiovascular disease, microalbuminuria)
- Surprisingly, the children of these children were also smaller

Cross-generational "*Epigenetic*" effect

- Confirmation required on other populations



EPIGENETIC MODIFICATIONS AT THE AGOUTI LOCUS AFFECTS COAT COLOUR

2 month old genetically identical Agouti mice

The Agouti coat colour gene is methylated

slightly	→ yellow
moderately	→ mottled
heavily	→ brown

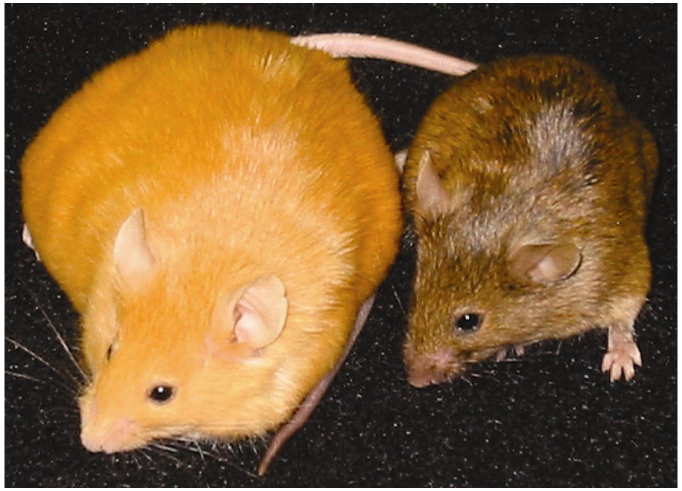
Maternal methyl donor dietary supplementation shifts the coat colour of the offspring from yellow to brown

Photo by Duke University Medical Center

Morgan et al. 1999 Nat Genet 23:314; Wolff et al. 1998 FASEB J 12:949

EPIGENETIC REGULATION OF GENES

1 year old genetically identical Agouti mice



Maternal methyl donor dietary supplementation
also reduces the incidence of obesity, diabetes, and cancer

Yellow dam → yellow offspring
Brown dam → brown offspring

Does transgenerational epigenetic inheritance occur in dairy cows?

Morgan et al. 1999 Nat Genet 23:314
Photo by Duke University Medical Center

DAIRY CATTLE RESEARCH

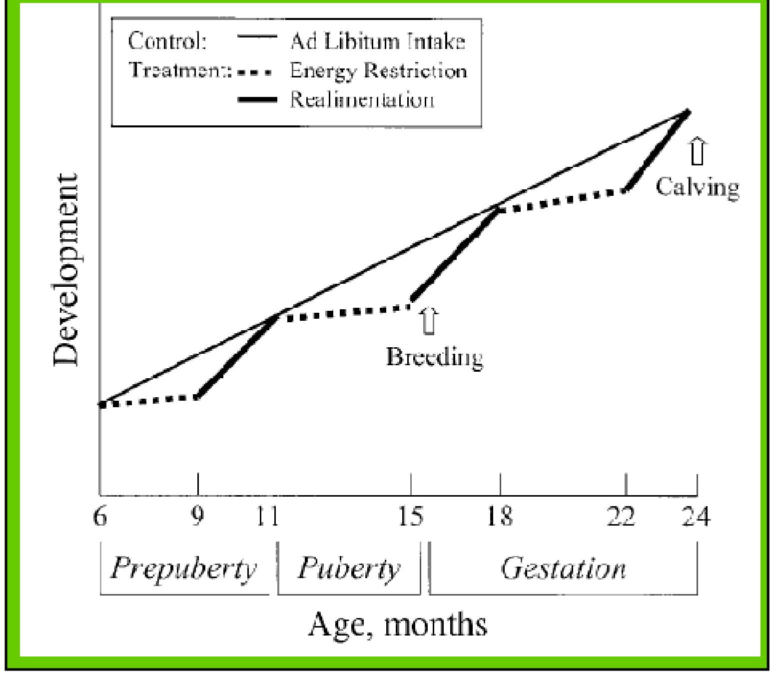
Nutrition influences

- mammary development and subsequent lactation
  Sejrsen et al. 1982 J Dairy Sci 65:793; Park et al. 1989 Growth Dev Aging 53:159
- and 2nd lactation
  Ford & Park, 2001 J Dairy Sci 84:1669

Hypothesis

A compensatory growth regime will enhance mammary development and subsequent milk production through epigenetic changes

Compensatory Growth Regime



Choi et al. 1998 J Nutr Biochem 9:380

↑ casein gene expression
↓ 5'-methyldeoxycytidine levels
in late gestation mammary tissue from heifers on test diet

DAM-DAUGHTER DAIRY COW RESEARCH
SEARCHING DATABASES

Berry et al. *2008 J Dairy Sci 91:329-337*
"Negative Influence of High Maternal Milk Production Before and After Conception on Offspring Survival and Milk Production in Dairy Cattle"

- Maternal milk production effects on daughter milk yields were small and negatively related
- High-producing cows tend to be in greater negative energy balance
- Thus, greater metabolic stress during pregnancy with subsequent negative effects on daughters' lactation performance

DAM-DAUGHTER STUDIES IN DAIRY COWS IN NZ
WORK IN PROGRESS

1. Statistical modelling of large dairy cow datasets
   - Jersey-Friesian crossbred trial
   - Sire Proving Scheme (SPS)

2. Pilot dam-daughter cow trial

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SEARCHING NEW ZEALAND DAIRY DATABASES

AIM

To determine if a dam's nutritional status
pre-conception and during pregnancy (trimesters 1, 2, 3)
has an effect on her daughter's subsequent milk production

Tissue energy stores = liveweight (kg) X [Tissue E]

HYPOTHESIS

Dam maternal environment during gestation influenced subsequent daughter phenotype, presumably by epigenetic modification *in utero*

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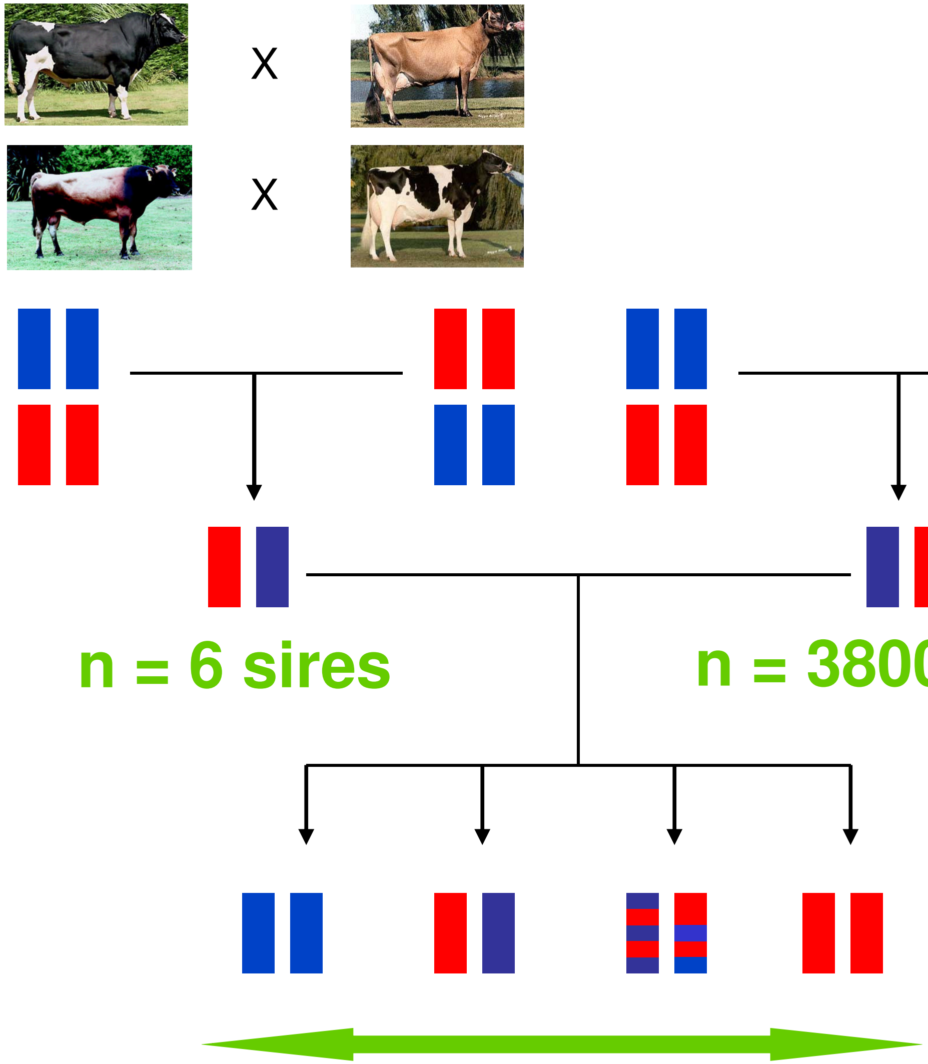
FRIESIAN-JERSEY CROSSBRED TRIAL (F2) DESIGN



Multiple dams

First cross (F1)

n = 6 sires

n = 3800 dams

Second cross (F2)

800 female F2 progeny

Spelman RJ *et al. 2004 New Zealand Society of Animal Production 64:92*

F2 PHENOTYPIC VARIATION

DAM - DAUGHTER PAIRS

F2 Dams (822 with full lactations)

Housed and managed together
Pasture based system
Herd test data
Daily milk recording and bodyweights
Biweekly body condition scores (BCS)

F3 Daughters (657 with herd test data)

Sold, housed on NZ commercial farms
Milk production and composition recording:
NZ national milk recording system

548 Dam-Daughter Pairs

- **Parentage**
- **Lactation Records**
- **Dam LWT and BCS Data**

WORK IN PROGRESS

1. **JxF Crossbred Trial**
   - Preliminary analysis using herd test data
     - **dam milk production during gestation was positively related to daughter's 1st lactation milk production**
   - Currently assessing dam's daily milk yield data
2. **NZ Sire Proving Scheme Data (LIC)**
   - Core of the NZ dairy sire genetic evaluation
   - Quality of records
     - 4 or more tests per lactation
     - > 20,000 records per year, last 10 years
   - Parentage reliability (dam and sire ID) accurate (~95%)
3. **USA datasets in collaboration with Prof Rich Erdman**

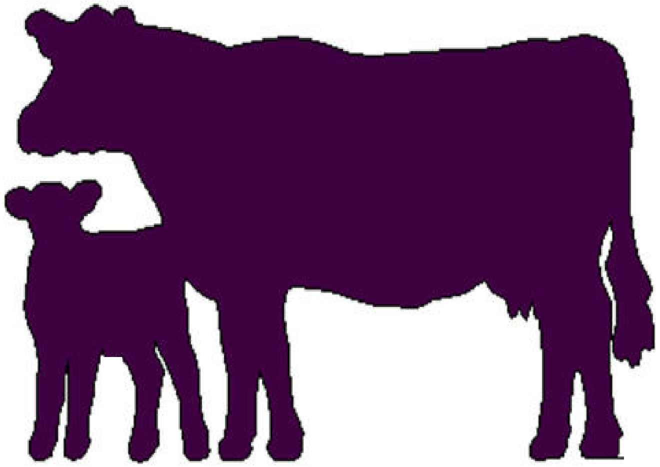
   Dam-Daughter, Dam-Daughter-Granddaughter effects
   Subsequent (2nd, 3rd, 4th, etc) daughter lactations

DAM-DAUGHTER EXPERIMENT: 2009/2010

AIM

To confirm preliminary results from crossbred trial database

dam milk production during gestation was strongly related to daughter 1st lactation milk production



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SELECTION OF HIGH AND LOW GROUPS

- Dam’s 2006 season herd test data available from AgResearch farm (milk yield, fat, protein)
- Heifers selected from dams with high or low milk production (lactation energy), n = 10/group
- 69 heifers due to calve 2009 from AgResearch farm

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MEASUREMENTS DURING DAUGHTER LACTATION

- **Daily milk yields**
  - Fitted Woods lactation curve (*Wood, 1967 Nature 216:164–165*)
  - $at^b e^{-ct}$ is defined by

    (a) Initial yield; (b) Ascent to peak; (c) Descent from peak and (t) time

    Calculate: Time to peak, Yield at peak, Persistency
- **Monthly milk samples**
  - Herd tests (Fat, Protein, Cell, Lactose)
  - PRL, IGF-1
- **Monthly blood samples**
  - PRL, IGF-1
- **Monthly BCS, liveweight**

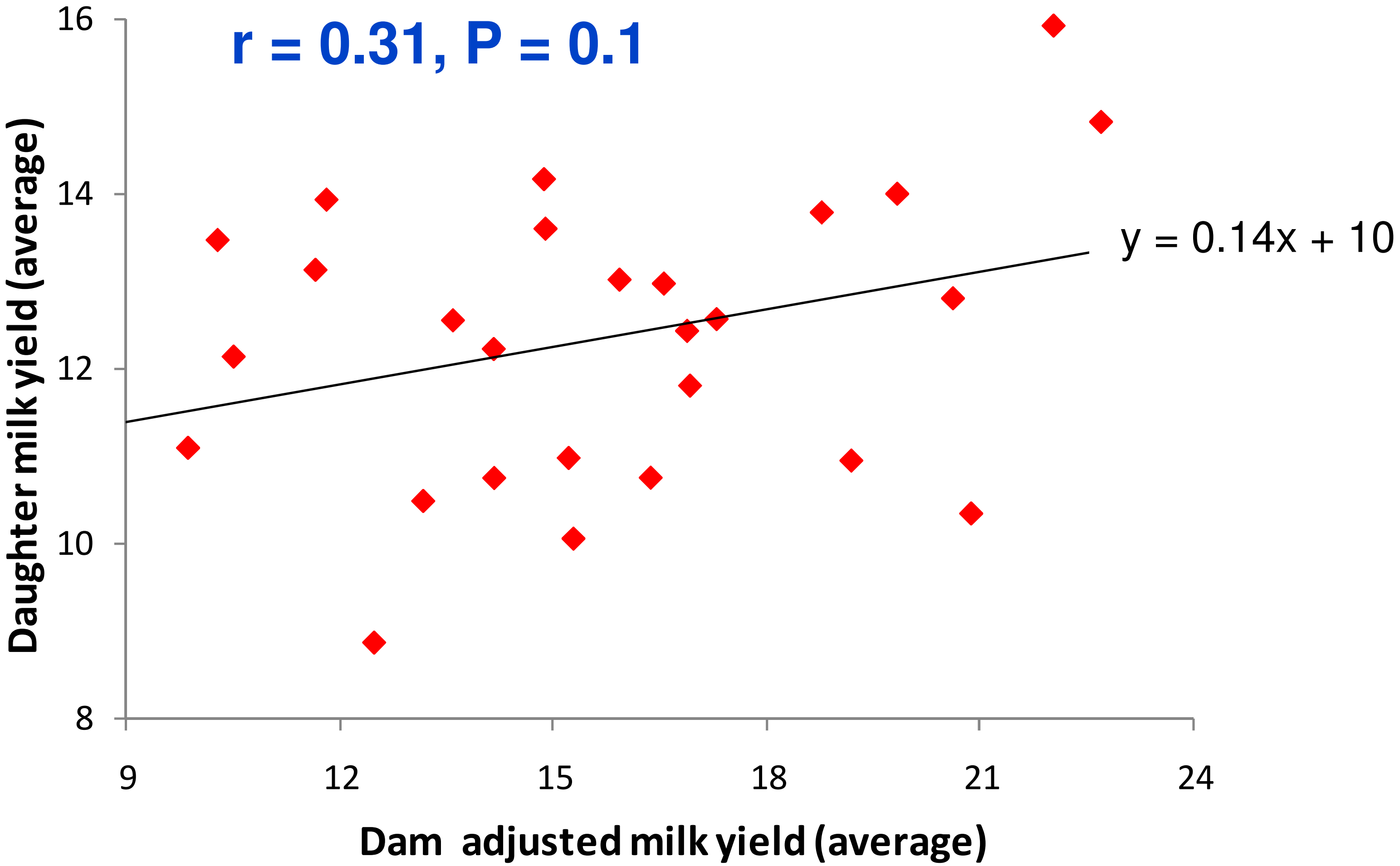
- **Mammary biopsies at mid and late lactation**
  - DNA methylation, gene expression, milk proteins & transcription factors

DAMS AND DAUGHTERS

Groups	Daughter BW	Dam BW	Dam age	Dam F16	Dam Lactation Days
high (n=9)	103	51	6.9	13.9	240
low (n=10)	108	36	4.4	12.0	234
sed	17	22	1.3	1.8	13
P value	ns	ns	P<0.1	ns	ns

Groups	Dam fat %	Dam protein %	Dam Lactation Energy Mcal	Average Dam 6 yr milk	Average Daughter milk
high (n=9)	4.8	3.6	3760	19.3	13.3
low (n=10)	5.1	3.9	2187	12.2	11.9
sed	0.3	0.2	192	0.9	0.7
P value	ns	P<0.1	**P<0.001**	**P<0.001**	**P=0.06**

RELATIONSHIP OF DAM AND DAUGHTER AVERAGE DAILY MILK YIELDS



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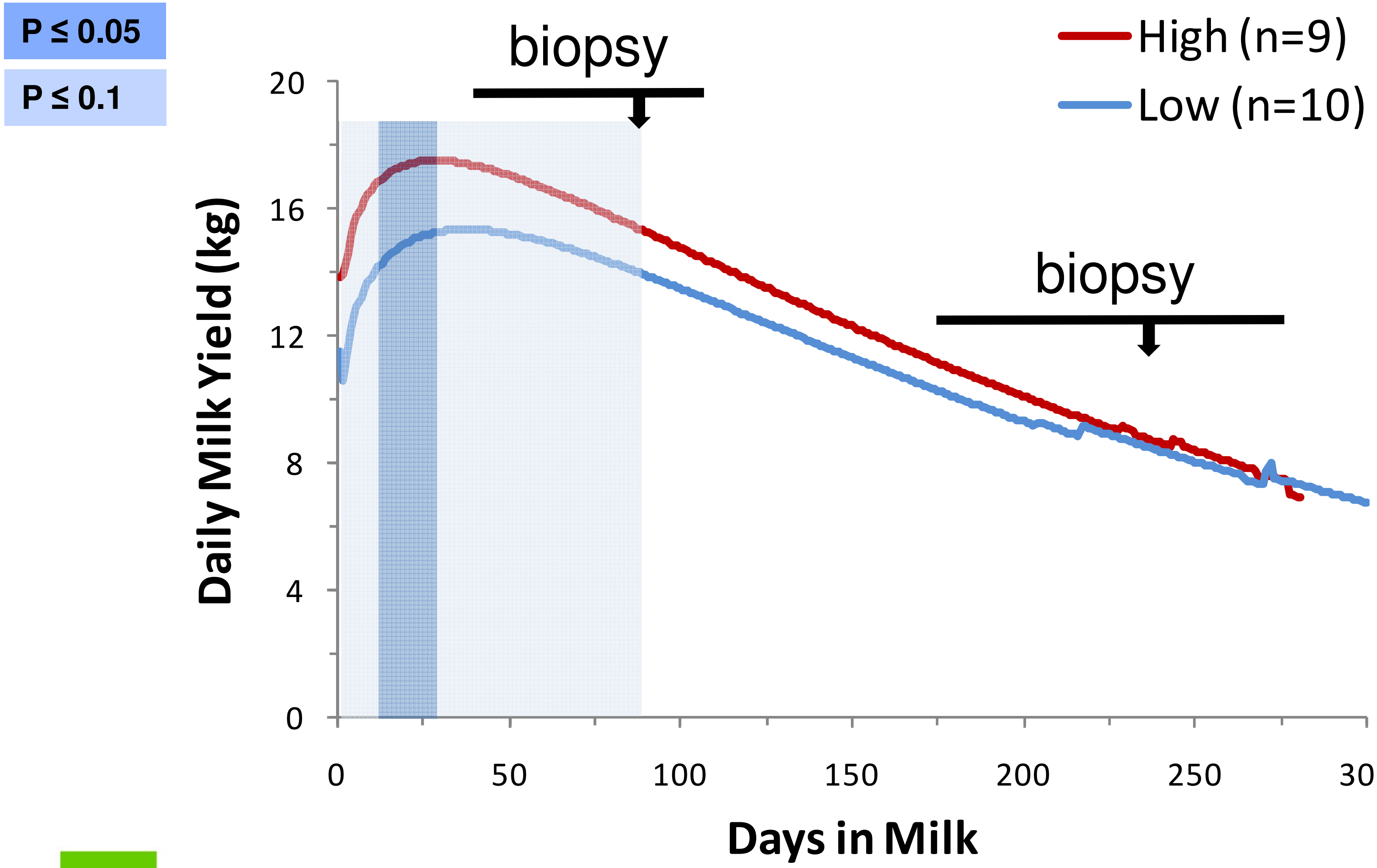
DAUGHTER'S DAILY MILK YIELDS



WORK IN PROGRESS

- **Milk yields differ at peak between heifers that were selected from high and low producing dams**

- **Analysis of biopsy samples**
    - **methylation analysis (sequenom, array)**
    - **mRNA analysis**

- Liveweights increased during lactation
- BCS measures unchanged throughout lactation
- Preliminary results - fat, protein, lactose, SCC, PRL, IGF-I measures are similar between the groups

SUMMARY

- There is currently no direct evidence for transgenerational epigenetic inheritance in dairy cows
- Limited indirect evidence suggests that the lactation performance of the dam has a strong influence on daughter's lactation performance
- This has been confirmed in a pilot dairy cow lactation trial
- We will examine if epigenetic mechanisms, such as DNA methylation play a role

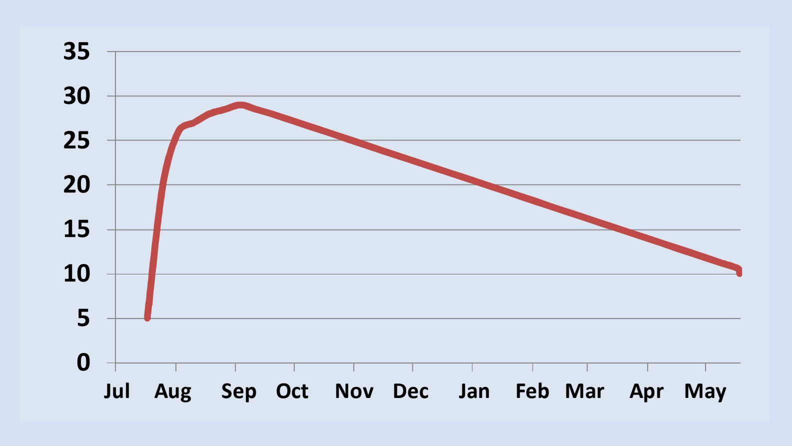
PART THREE

- Introduction - Epigenetic mechanisms
- Transgenerational epigenetic inheritance
- **Dynamic epigenetic mechanisms**

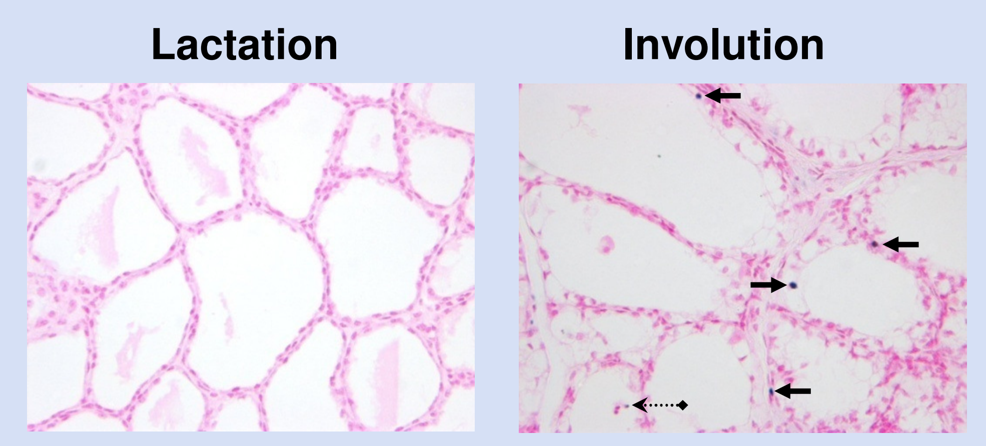
DNA METHYLATION IS ASSOCIATED WITH BOVINE αS1-CASEIN GENE EXPRESSION DURING MAMMARY INVOLUTION

MILK YIELD LOSSES IN DAIRY COWS

- Gradual involution occurs following peak lactation
- Once-daily milking compared to twice-daily
- Apoptotic death of mammary epithelial cells



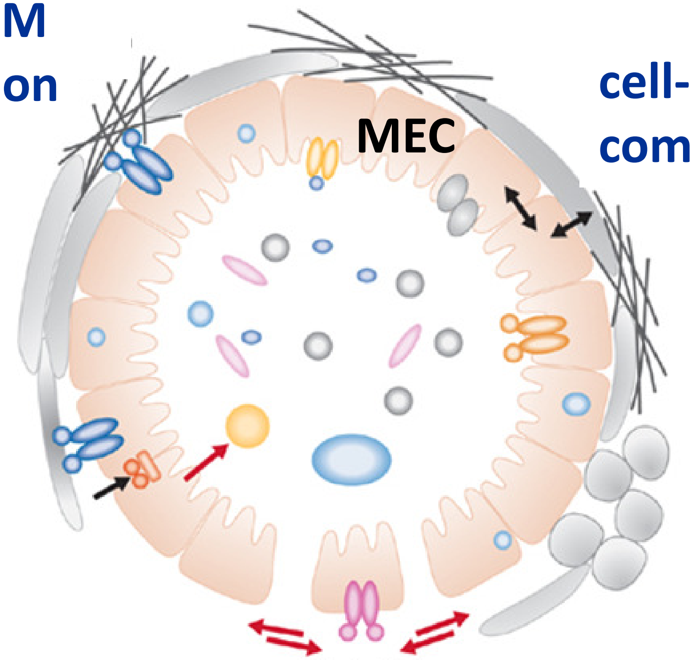
Typical lactation curve



Apoptotic death of mammary epithelial cells

OBJECTIVE

In bovine, what are the molecular mechanisms within the mammary epithelial cells responsible for the switch from lactation to non-lactation?



cell-ECM communication

MEC

cell-cell communication

Mechanical stress

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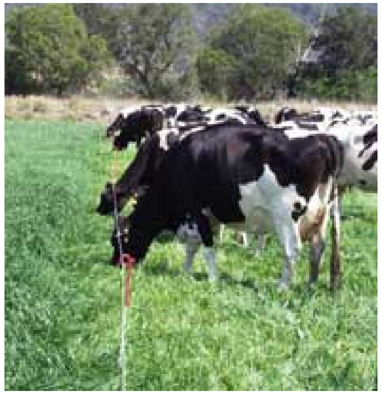
TRIAL DESIGN

INDUCED INVOLUTION OF BOVINE MAMMARY GLAND

Season 1: 0, 6, 12, 18, 24, 36, 72h (n=6/group) post milking
Season 2: 72h (n=4) and 8 days (n=6) post milking

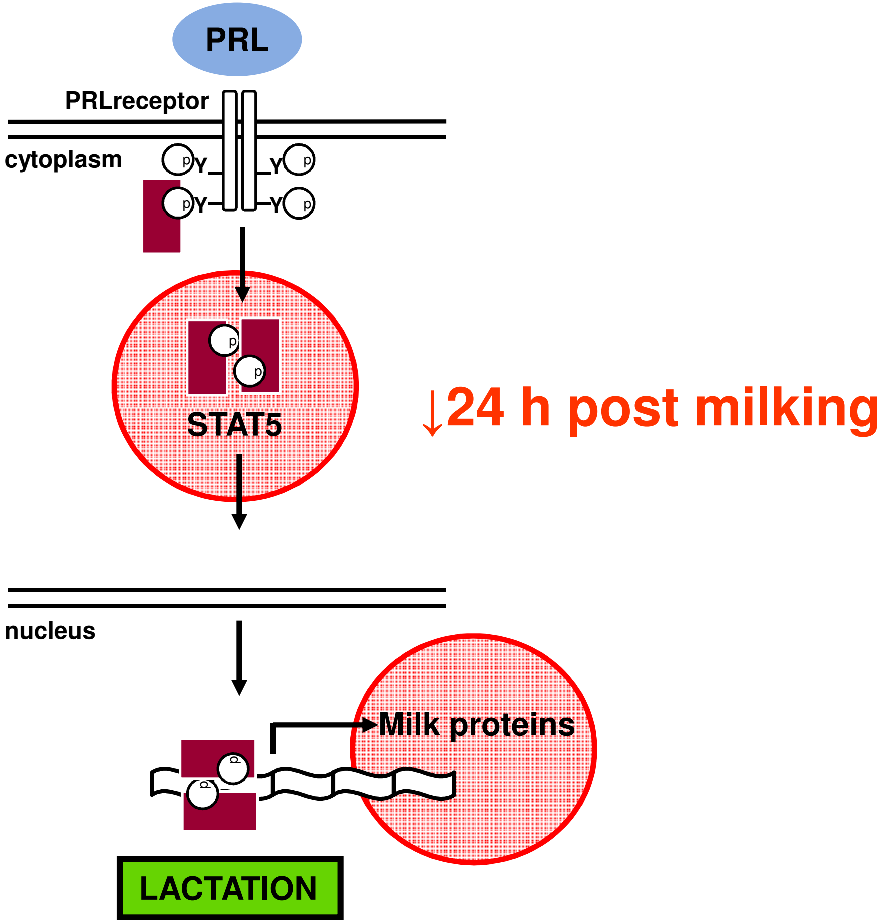
- Friesian heifers
- Non-pregnant
- Mid lactation
- Low SCC
- Pasture-fed
- Ave milk yield = 14.3 ± 0.3 kg/d



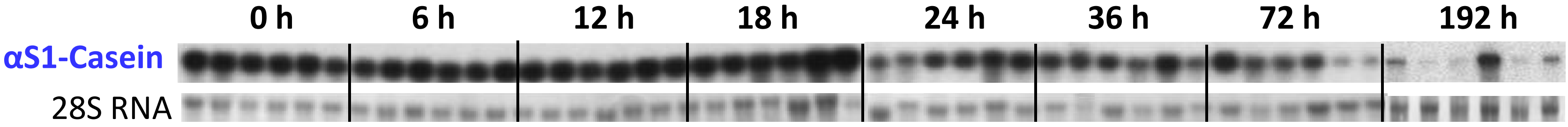
Alveolar mammary tissue collected

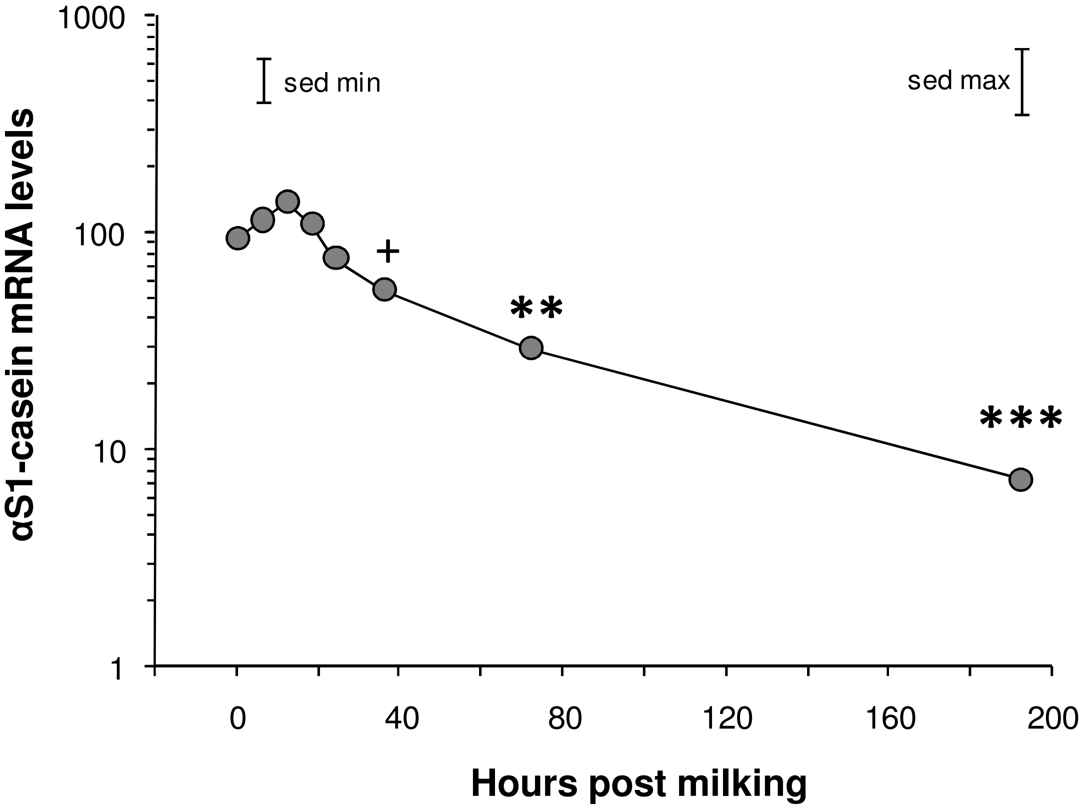
- αS1-casein
  - Real-time RT-PCR
  - SDS-PAGE analysis
  - Methylation profiling
    - Quantitative methylation analysis by Sequenom
    - Determines % of 5methyl-cytosine in DNA

PROLACTIN SIGNALLING IN THE BOVINE MAMMARY GLAND

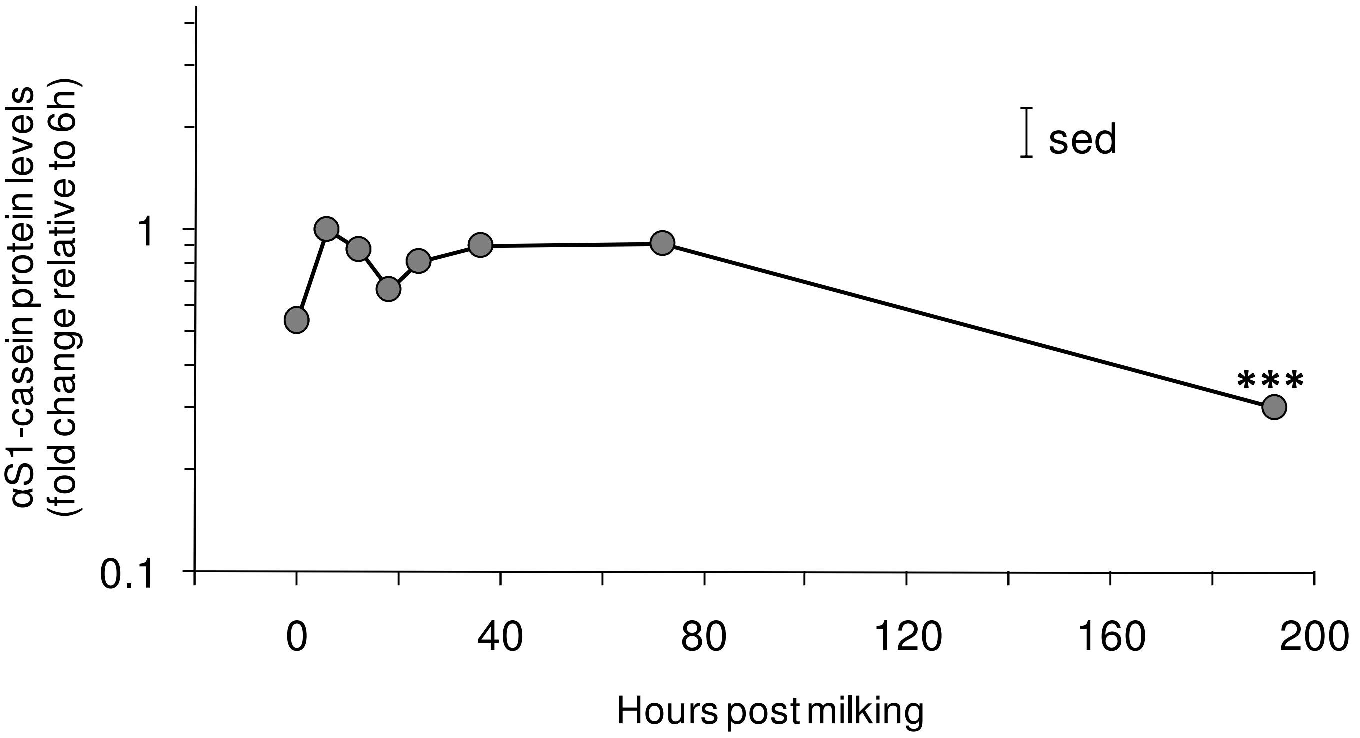


DOWN-REGULATION OF MAJOR MILK PROTEIN GENES IS CHARACTERISTIC AND EARLY EVENT

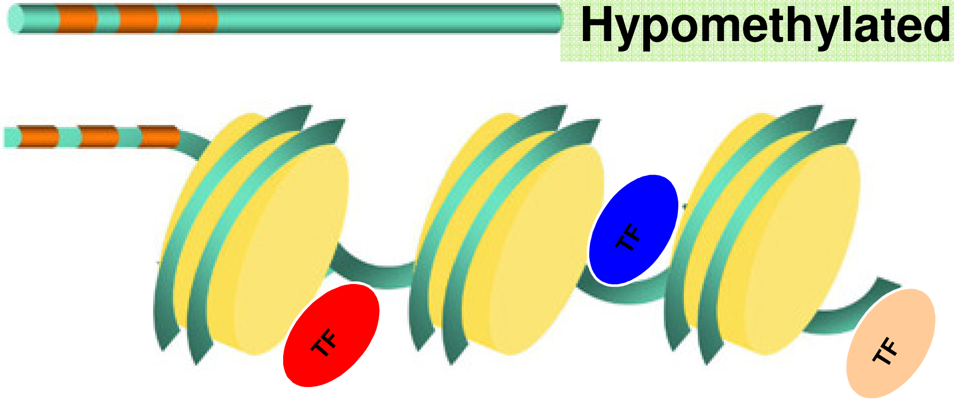




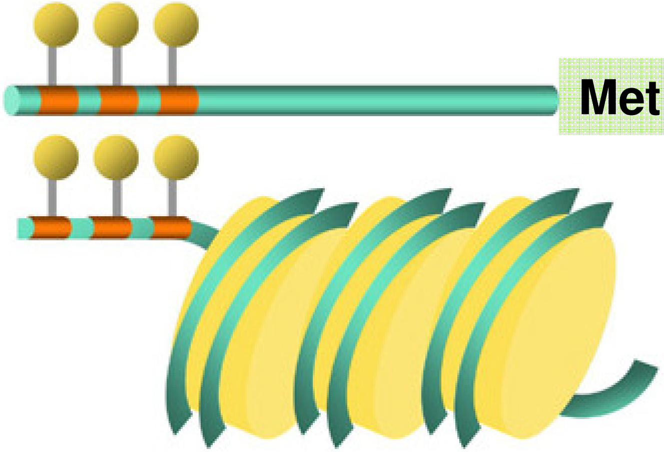
SDS-PAGE ANALYSIS OF αS1-CASEIN PROTEIN LEVELS



DNA METHYLATION AND GENE SILENCING



Hypomethylated DNA is open and active



Methylated DNA is condensed and silent

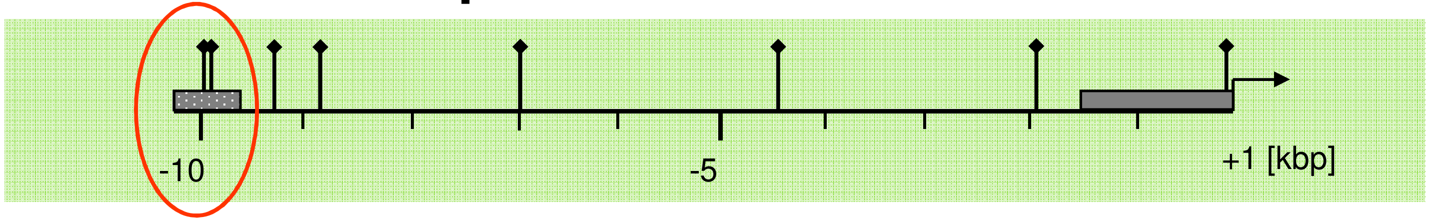
EPIGENETIC MECHANISMS IN BOVINE MAMMARY GLAND

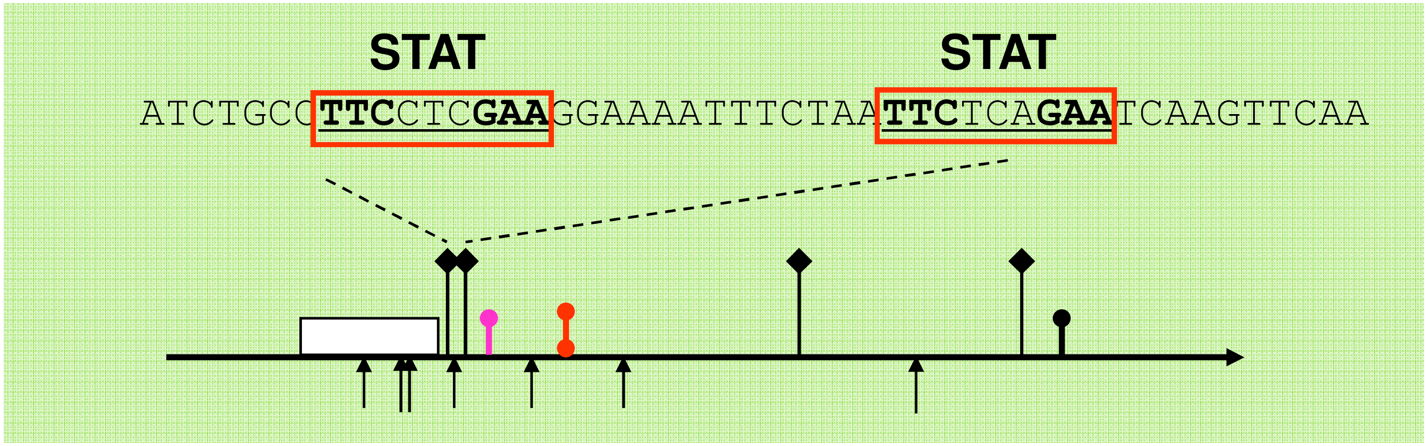
Vanselow et al. 2006 DNA-remethylation around a STAT5-binding enhancer in the αS1-casein promoter is associated with abrupt shutdown of αS1-casein synthesis during acute mastitis. *Mol Endocrinol*

Is methylation in milk protein gene promoters associated with the down-regulation of expression during involution?

STAT-SITES IN THE αS1-CASEIN PROMOTER

STAT-binding sites



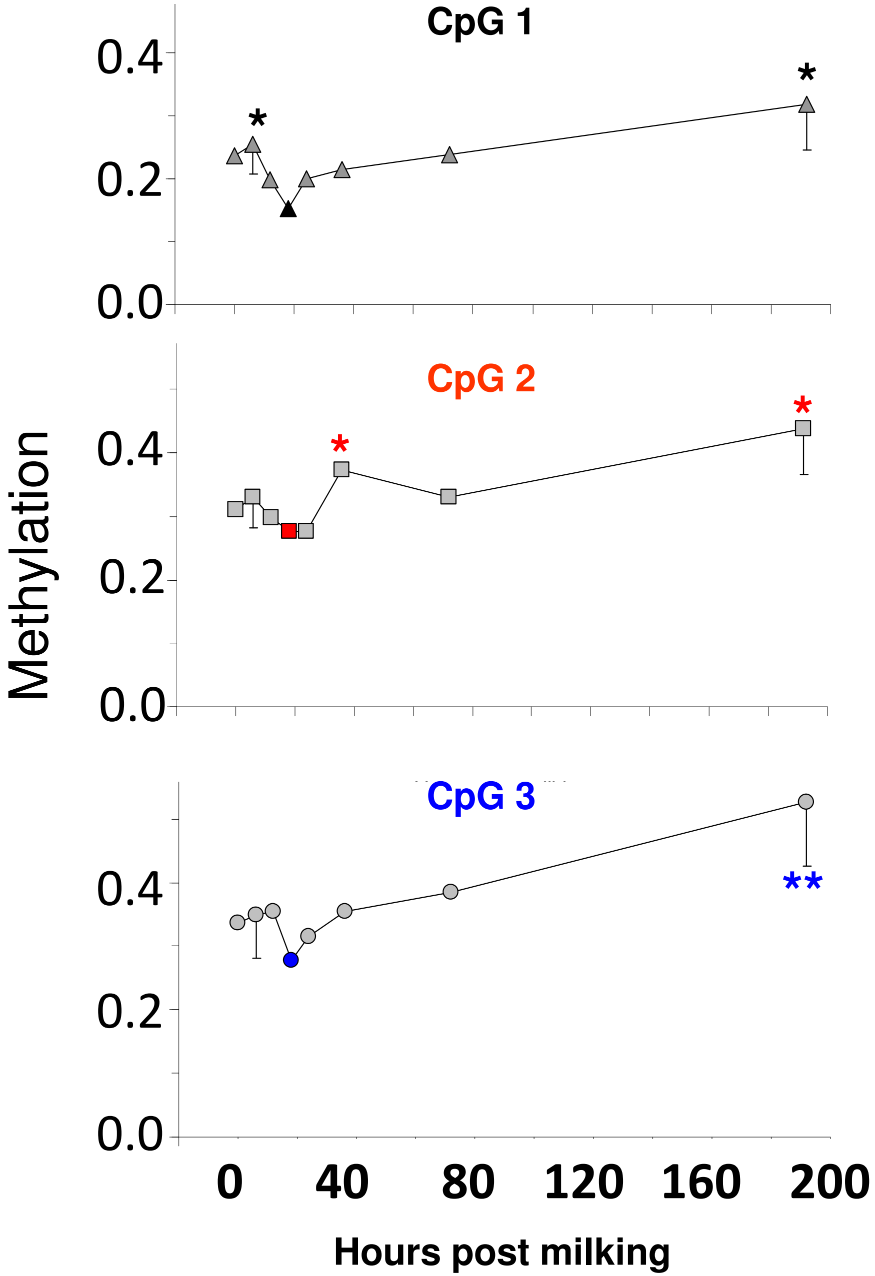


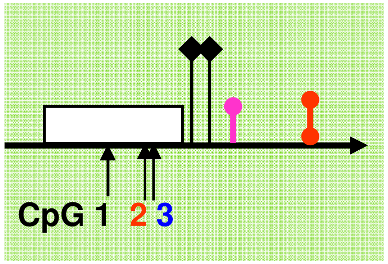
CoRE (composite response elements)**: STAT, C/EBP, GR**

CG-dinucleotide

Vanselow et al. 2006 Mol Endocrinol

METHYLATION OF DISTAL CORE IN αS1-CN PROMOTER





Methylation

- ↑ during involution
- Negatively correlated with αS1-CN mRNA

DNA Compaction

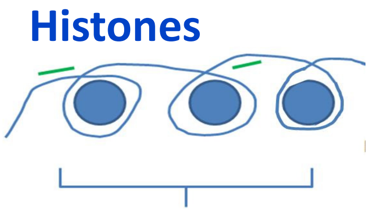
Mastitis

5 non-pregnant Friesian cows in mid-late lactation
Infused 2 teats/cow with 1000–1500 cfu *strep. uberis*

Clinical signs of mastitis
Mammary alveolar tissue was collected at slaughter
Infected and control quarters

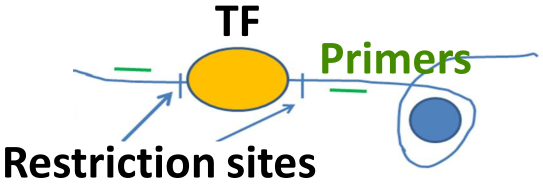
Chromatin accessibility assay (CHART)

- Measures degree of DNA compaction
- Isolate nuclei, restriction digest, proteinase K treat
- Real time PCR, primers flanking distal CoRE STAT5-binding site & Dde1 RE site
- Expressed as % DNA protected



Histones

Protected
NOT CUT
PCR product



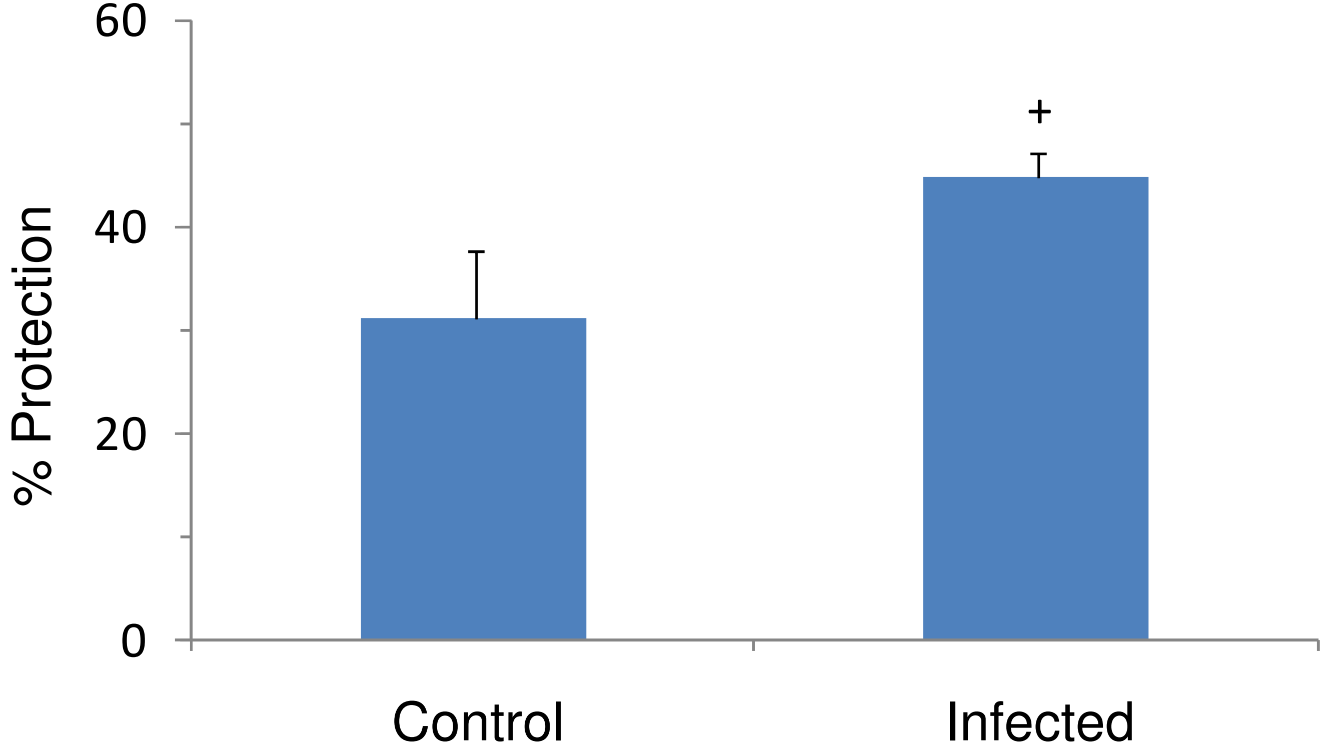
TF

Primers

Restriction sites

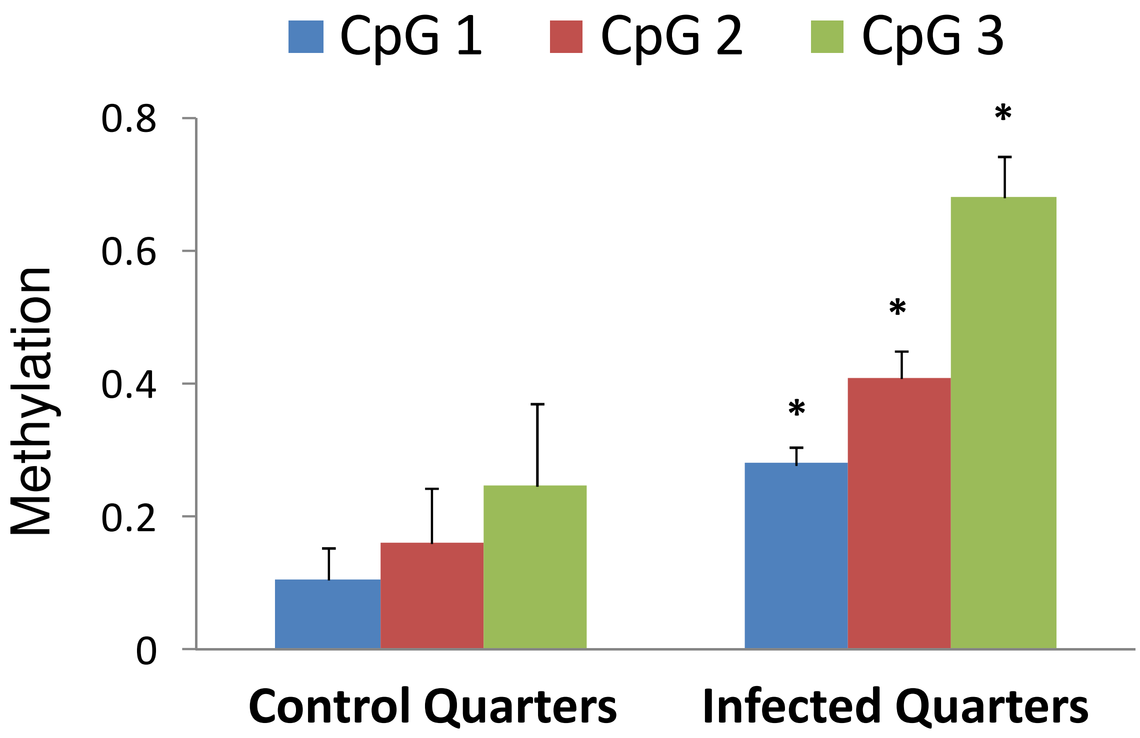
Open
CUT
No PCR product

DNA COMPACTION OF αS1-CN DURING MASTITIS



The DNA compaction of casein ↑ by 14% ± 7%
during infection from 31% to 45%

METHYLATION OF DISTAL CORE IN αS1-CN PROMOTER



- In mastitic samples, methylation ↑ at the ~10Kb upstream functional STAT5-binding site compared to non-mastitic
- Negatively associated with αS1-casein mRNA

CONCLUSIONS

In bovine mammary alveolar tissue, αS1-casein

- ↓ mRNA by 36 h post milking
- ↓ protein by 8 days post milking
- ↑ methylation CpG 2 by 36 h and all 3 CpGs at the distal CoRE by 8 d post milking compared to 18 h

- There appears to be a transient decrease in methylation at 18 h coinciding with
  - maximum alveolar engorgement
  - tight junction leakiness
  - decreased mammary blood flow

18 h may be a key time point in switching between a lactating and non-lactating phenotype

It is possible that DNA methylation may play a role in this

DYNAMIC DNA METHYLATION MECHANISMS

RE-INITIATION OF LACTATION COW TRIAL

- Friesian heifers
- 95 DIM
- Infection free
- 6 groups (n=6 per group) - 5 per group slaughtered

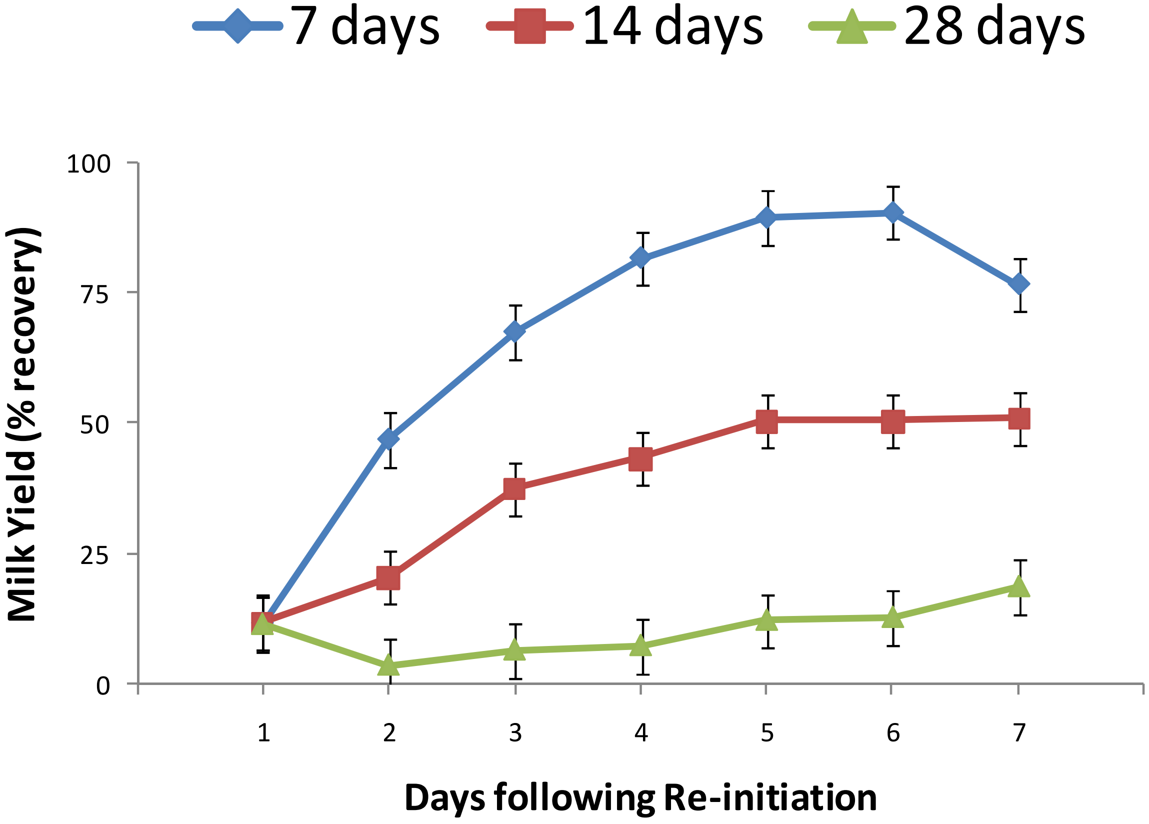
  A: Control cows slaughtered at mid lactation
  B: 7 days non-milking
  C: 7 days non-milking, 7 days milking
  D: 14 days non-milking, 7 days milking
  E: 28 days non-milking
  F: 28 days non-milking, 7 days milking

- Milk yield, milk composition, SCC
- Milk samples
- Blood samples

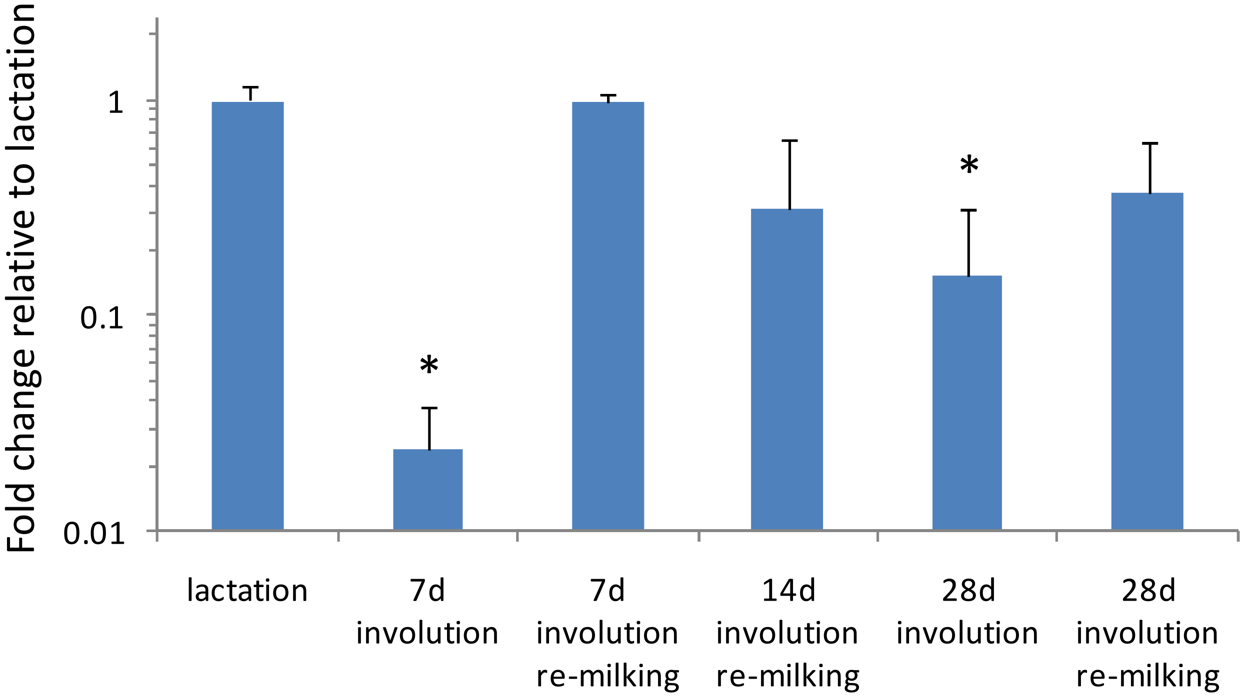
} Prior and during trial, PRL and IGF-I

- Mammary alveolar tissue at slaughter
  - Flash frozen tissue
  - Histology

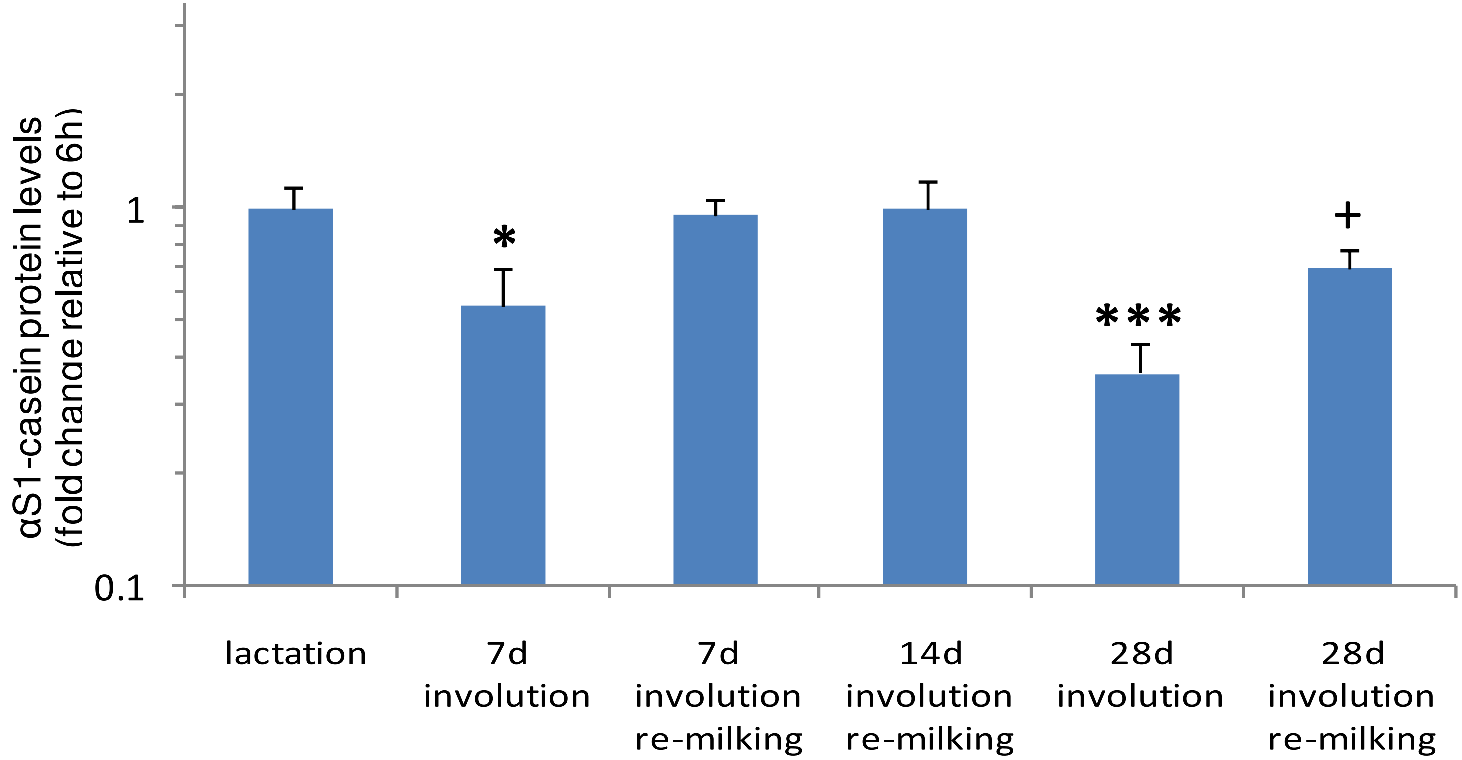
MILK YIELD % RECOVERY



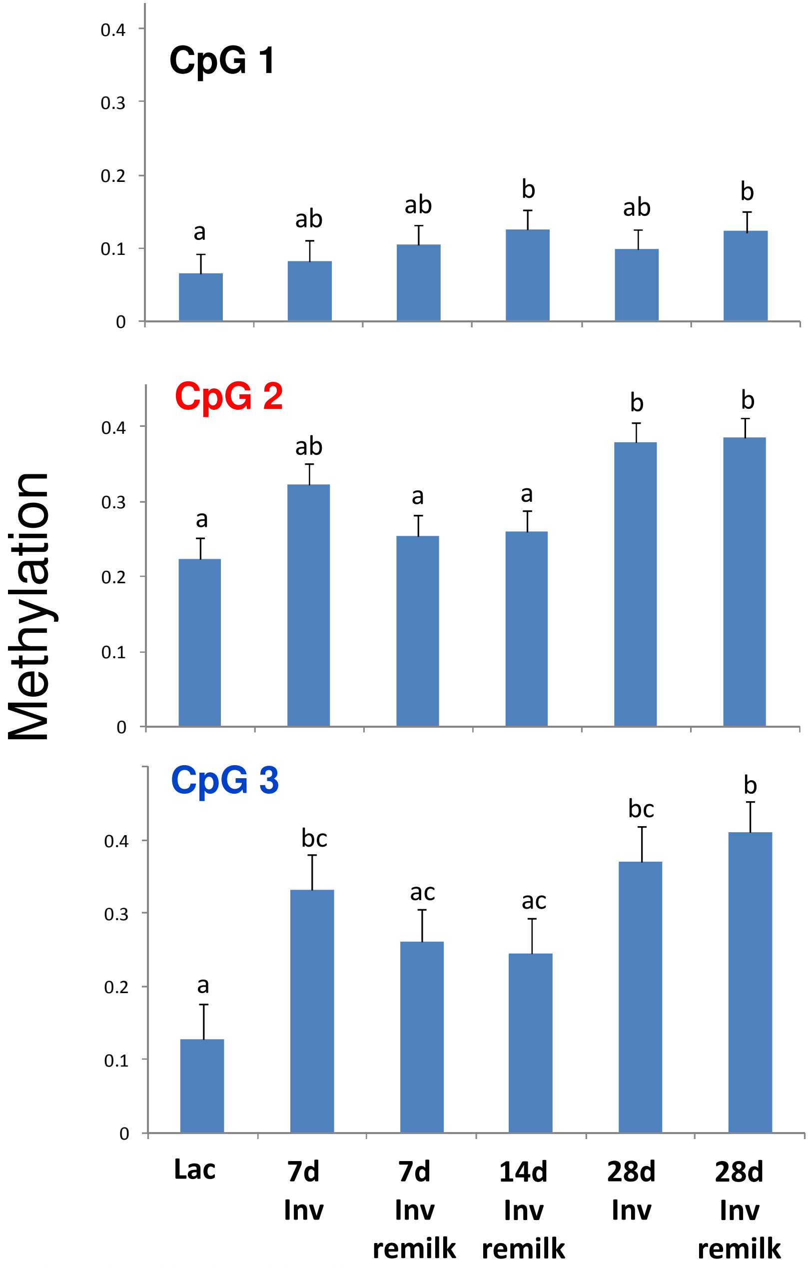
αS1-CASEIN mRNA LEVELS

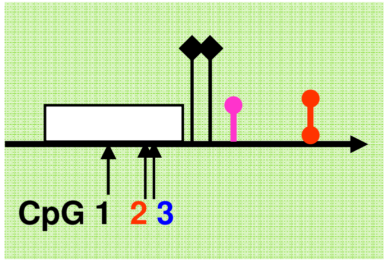


αS1-CASEIN PROTEIN LEVELS



METHYLATION OF THE DISTAL CORE IN THE αS1-PROMOTER





Methylation:

- ↑ in the αS1-CN promoter during involution
- Reversible after 7 d non-milking
- Irreversible after 28 d non-milking
- Negatively correlated with mRNA

PRELIMINARY CONCLUSIONS

- Milk yields recover 90%, 50% and 20% following re-initiation of milking after 7, 14 and 28 days non-milking periods
- αS1-casein mRNA and protein
  - ↓ 7 and 28 days involution compared to lactation
- αS1-casein distal CoRE methylation levels
  - ↑ 7 and 28 days involution compared to lactation
  - Reversible after 7 d non-milking
  - Irreversible after 28 d non-milking
- Currently analysing methylation status of different milk protein genes

When lactation is almost fully recoverable → methylation levels are reversible

DNA methylation may play a dynamic role in regulating milk protein gene expression

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SUMMARY

- The transgenerational epigenetics story:
  - Very limited information
  - Further analysis of large databases required
  - Animal trials involving induced changes in dam gestational milk required
- Dynamic changes in methylation demonstrated for regulating milk protein gene expression
- Novel approaches to enhancing the life-time lactation performance of the dam and also her off-spring

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AGRESEARCH TEAM

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