Epigenetic Differences of Dairy Cows Classified with Immune Response Traits

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Recipient of 2014 ADSA-EAAP Travel Award sponsored by Novus International & ADSA

UNIVERSITY of GUELPH
CHANGING LIVES IMPROVING LIFE

Never start a presentation with a graph…

Never start a presentation with a quiz!!

Identical or Not Identical?

Identical or Not Identical?

Epigenetics in the Press

Balancing act: Cell senescence, aging related to epigenetic changes

Researchers Discover an Epigenetic Lesion in Hippocampus of Alzheimer's

Jan. 21, 2014 — New research demonstrates, for the first time, the

ONLY HUMAN: December 1, 2013

Mice Inherit Specific Memories, Because Epigenetics?

by Virginia Hughes

reveals how the choices you make can change your genes

Epigenetics: New Link Between Nutrition, Cancer

Jan. 9, 2014 — In a recent article, researchers explore the possible effects that diet can have on gene expression through epigenetic mechanisms. Explaining the impact of

FROM THE MAY 2013 ISSUE

Grandma's Experiences Leave a Mark on Your Genes

Your ancestors' lousy childhoods or excellent adventures might change your personality, bequeathing anxiety or resilience by altering the epigenetic expressions of genes in the brain.

By Dan Hurley | Tuesday, June 11, 2013

Epigenetics of Breast Cancer Family History

Jan. 7, 2014 — Researchers have examined whether a specific epigenetic modification (more specifically, methylation of the DNA) can be associated to breast

shape the rest of your life

Why Your DNA May Not Be Your Destiny

Denise Chow, LiveScience Staff Writer | June 04, 2013 12:52pm ET

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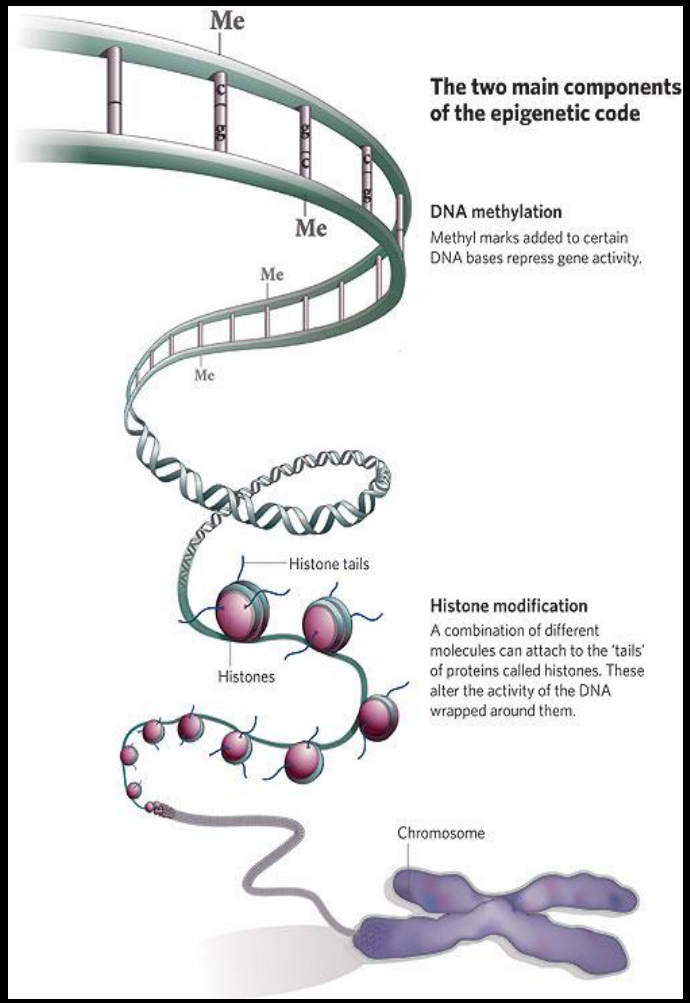
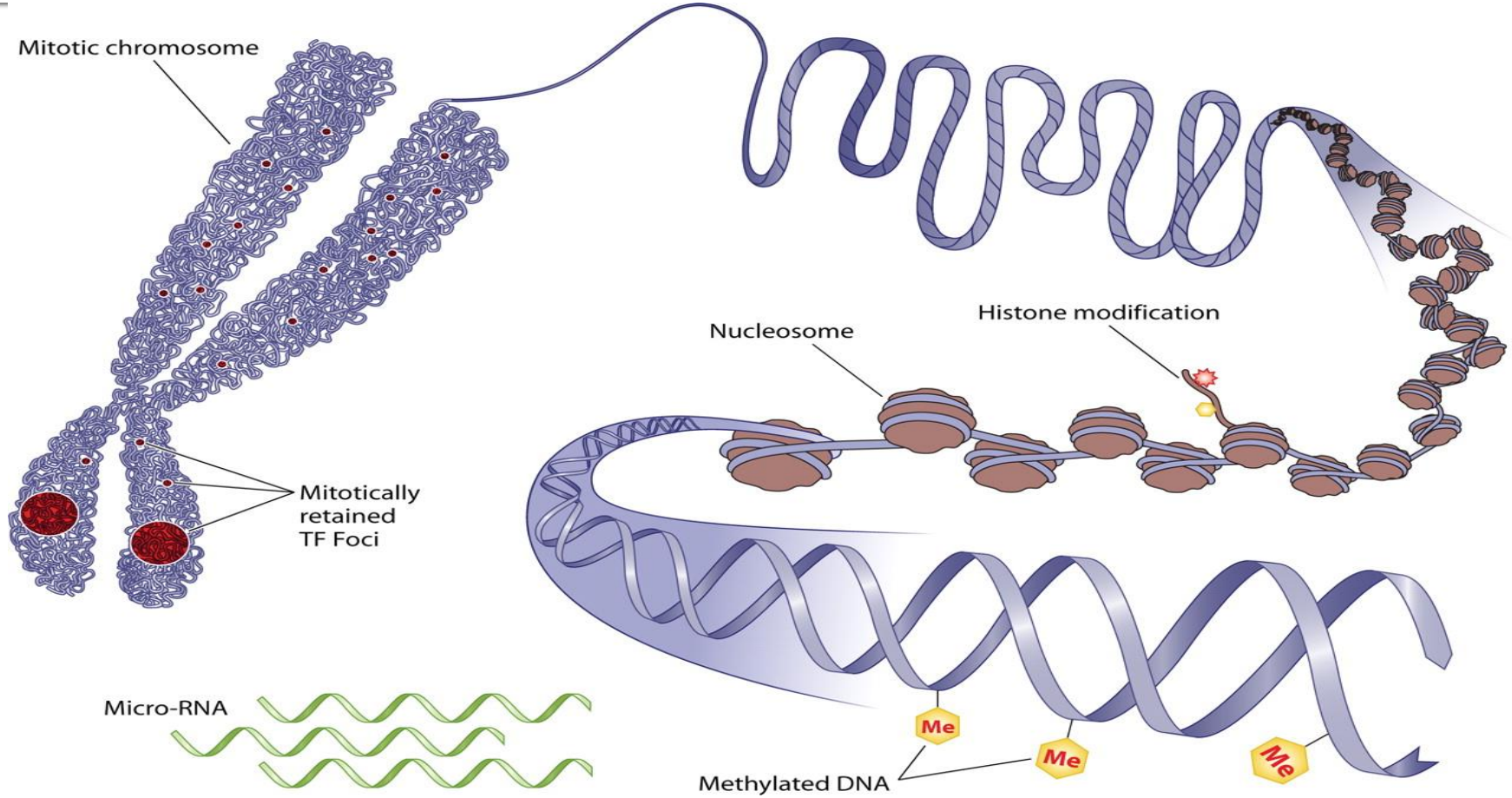
Epigenetics



- "above" genetics – gene and environment interaction
- Acts like a switch
  - turning genes on and off
- Genomics = the blueprints
- Epigenetics = the engineers

Zaidi S K et al. Mol. Cell. Biol. 2010;30:4758-4766

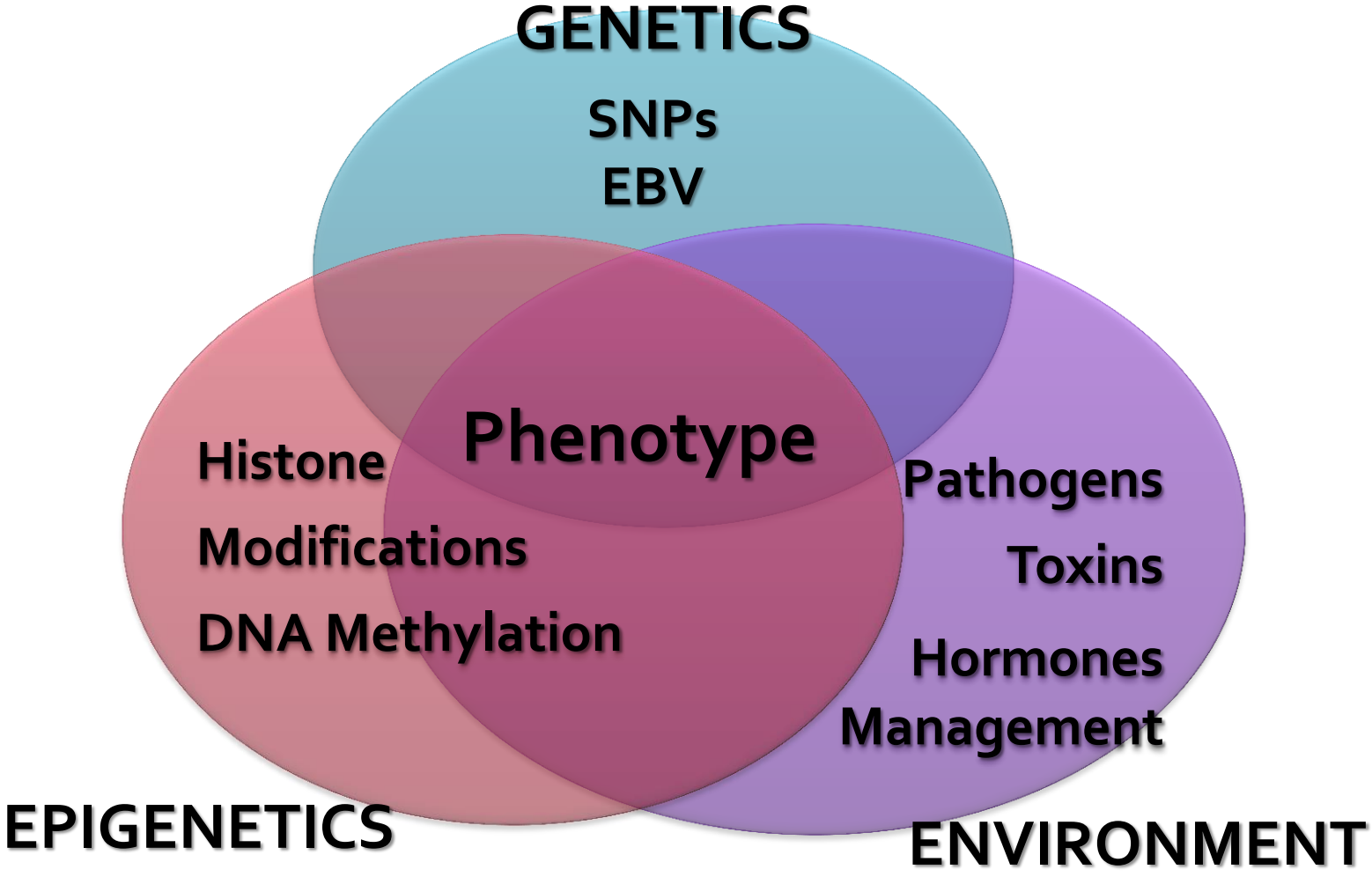
Mechanism of Epigenetics



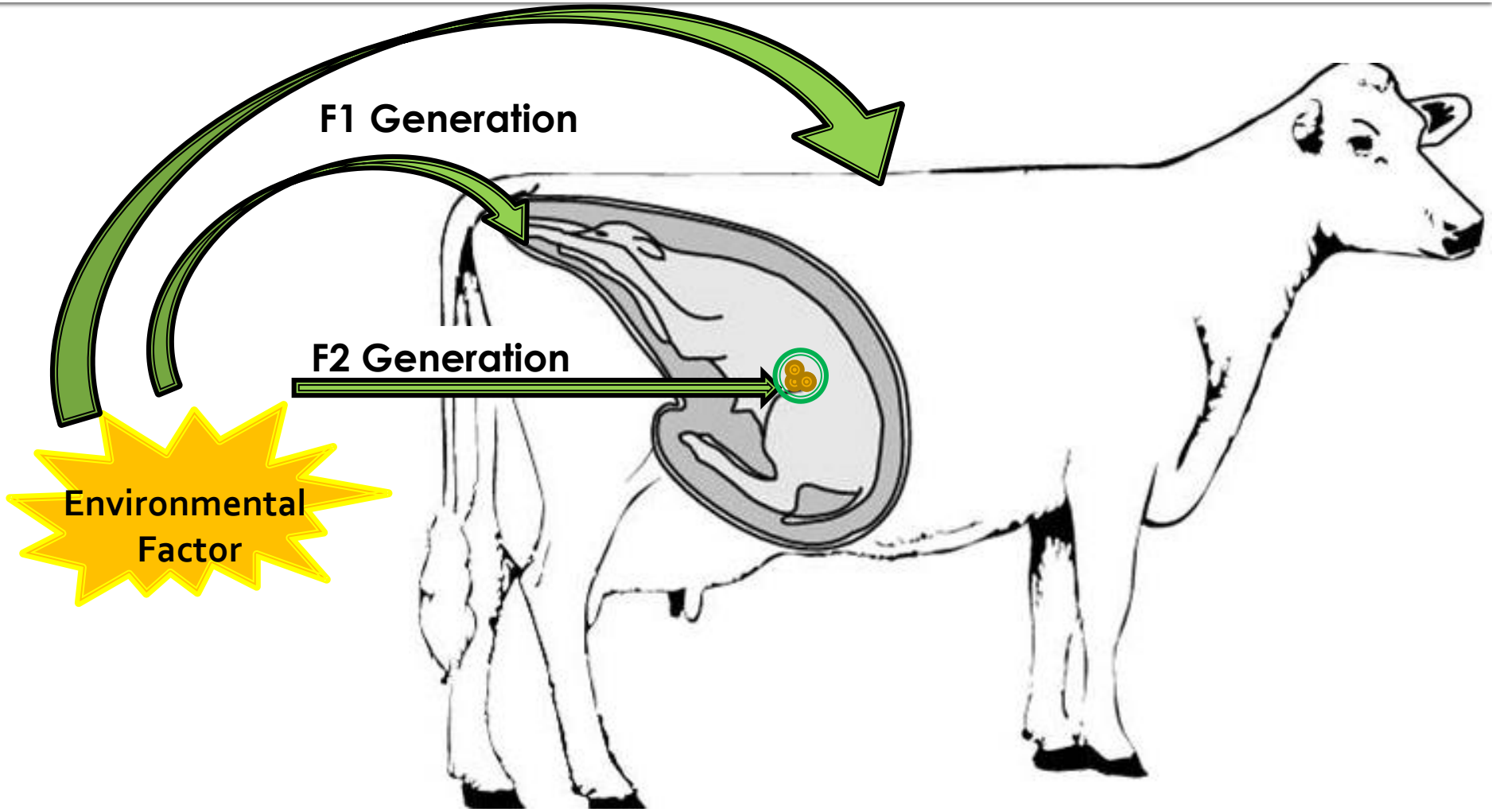
Zaidi S K et al. Mol. Cell. Biol. 2010;30:4758-4766

Molecular and Cellular Biology

Epigenetics Role in Animal Health



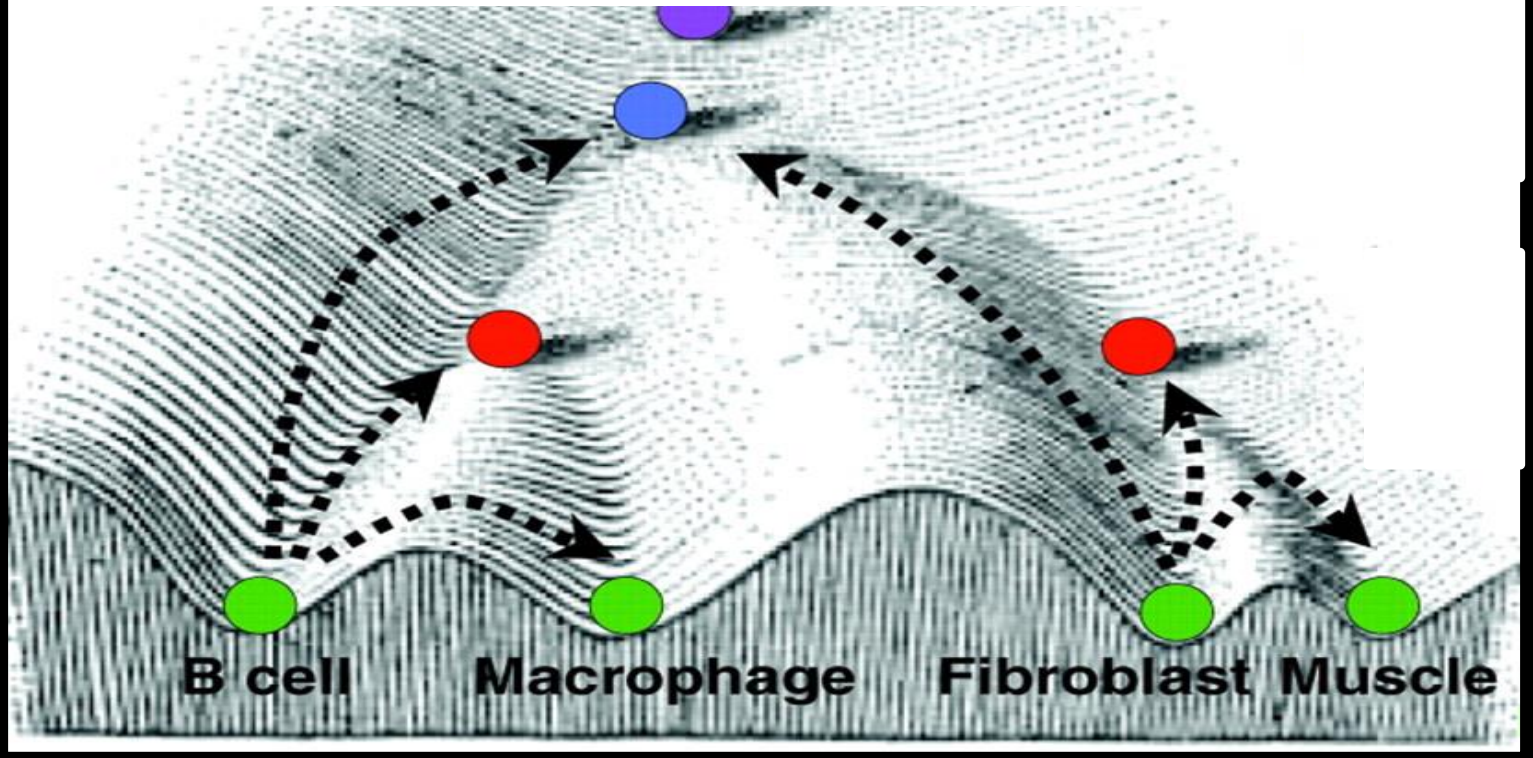
Environmental Influences Over Generations



The Epigenetic Landscape

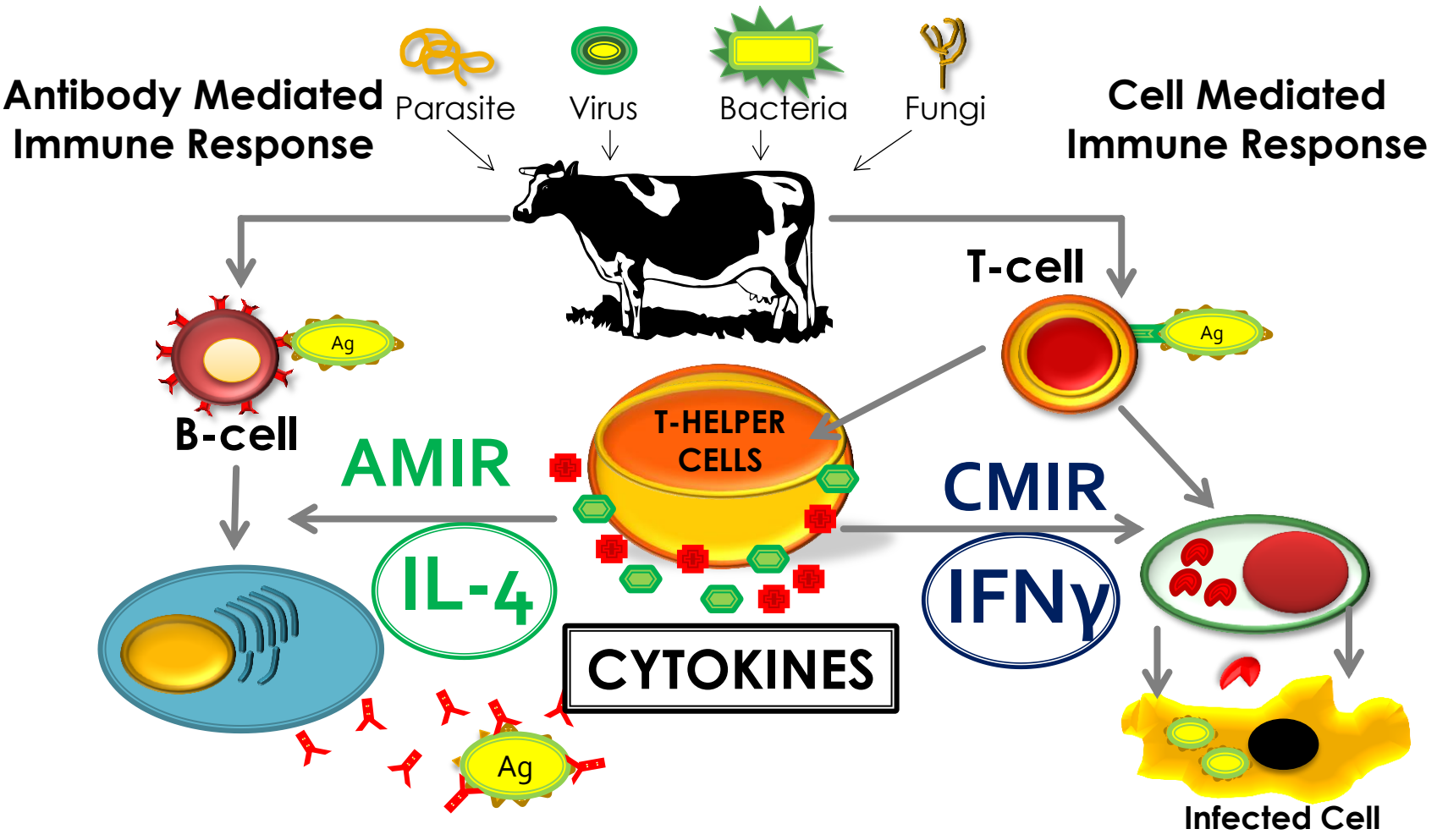
↑ DNA Methylation turns **OFF** genes

↓ DNA Methylation turns **ON** genes



Hochedlinger K , Plath K Development 2009;136:509-523

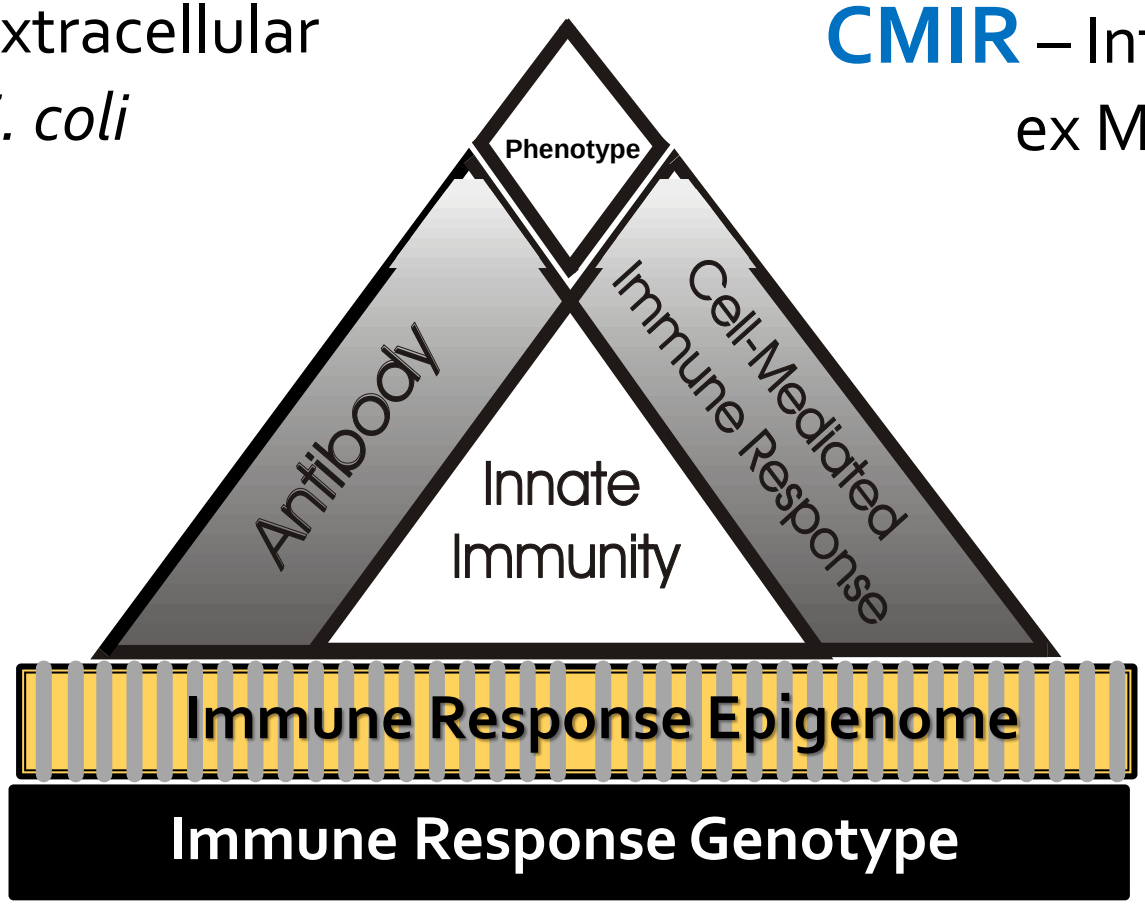
Biased Immune Responders Cows



The Immune System

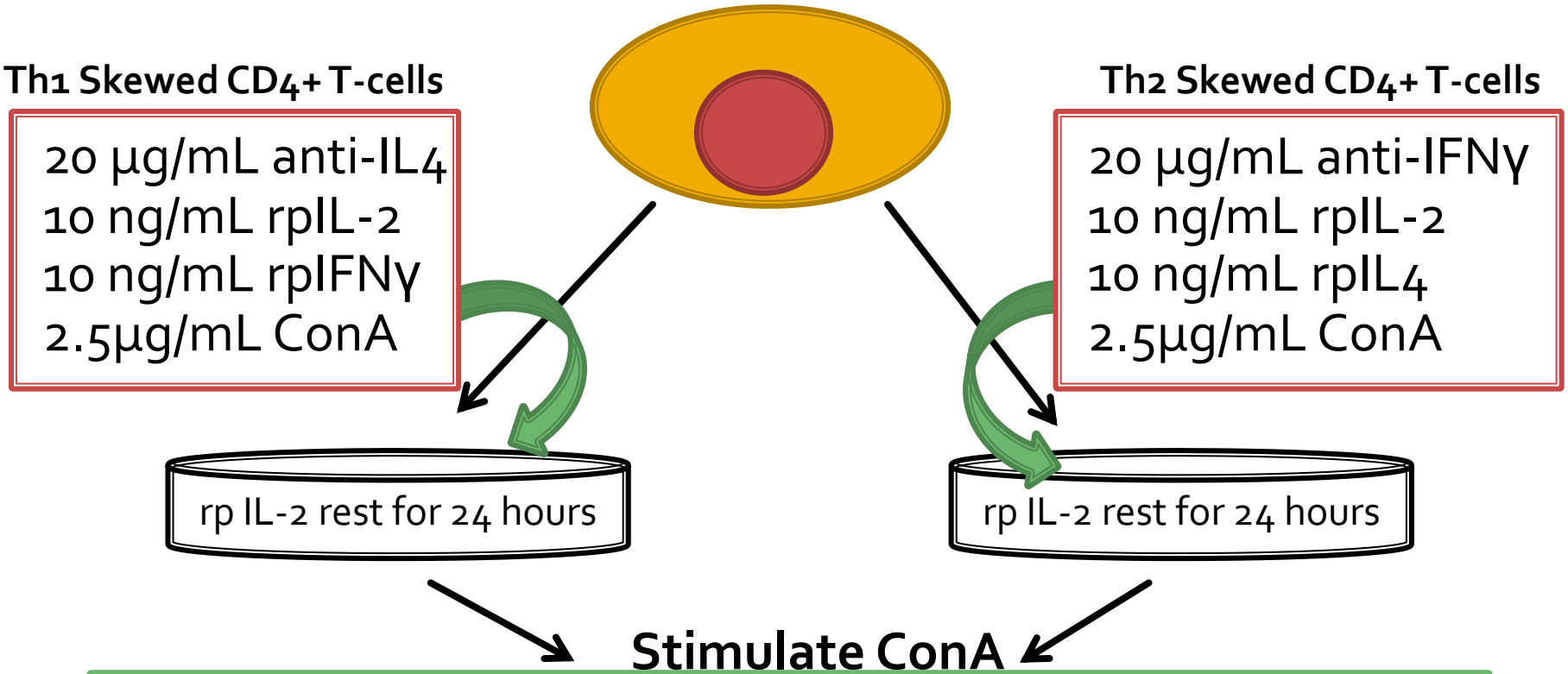
AMIR – Extracellular
ex *E. coli*

CMIR – Intracellular
ex MAP



Wilkie, B. and Mallard, B. 1999 *Vet Immunol Immunopath* 72:231-235

Generation of Bovine Type 1 and Type 2 Skewed CD4+ T-cells



Harvest:

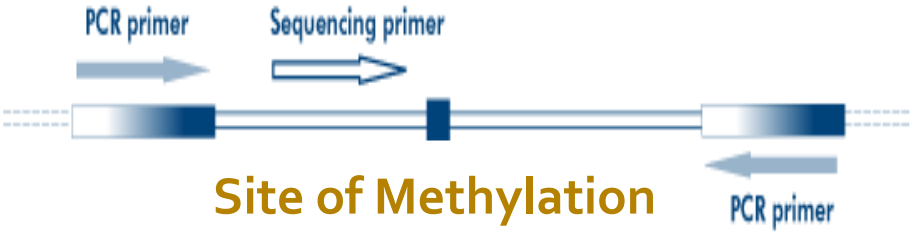
- Cell culture supernatant for measuring cytokines (ELISA)
- RNA for quantifying transcript (Realtime PCR)
- DNA for methylation profiling (Bisulfite Conversion Assay)

Bisulfite Pyrosequencing

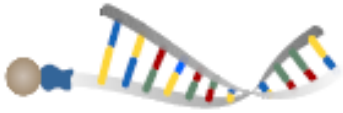
Magnetic bead Isolated T-Helper Cells

Extract DNA

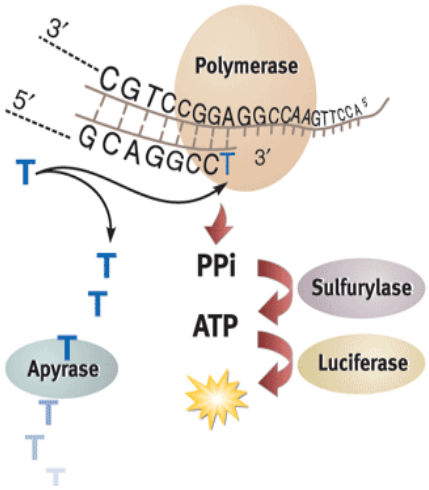
Treat with Bisulfite

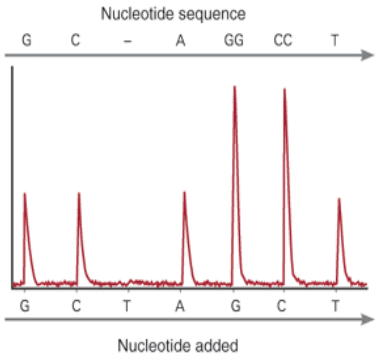


PCR Product Capturing

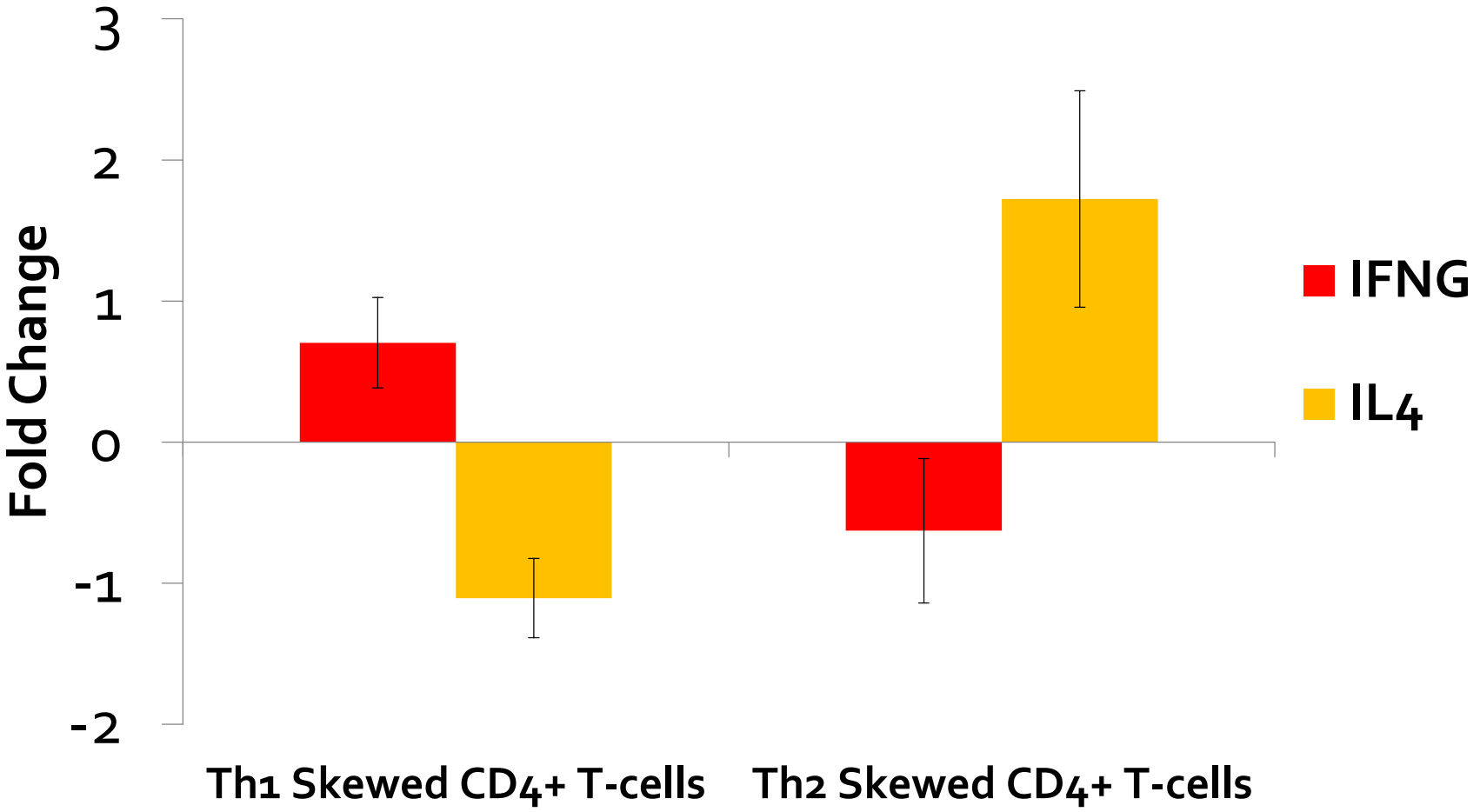


Pyrosequencing



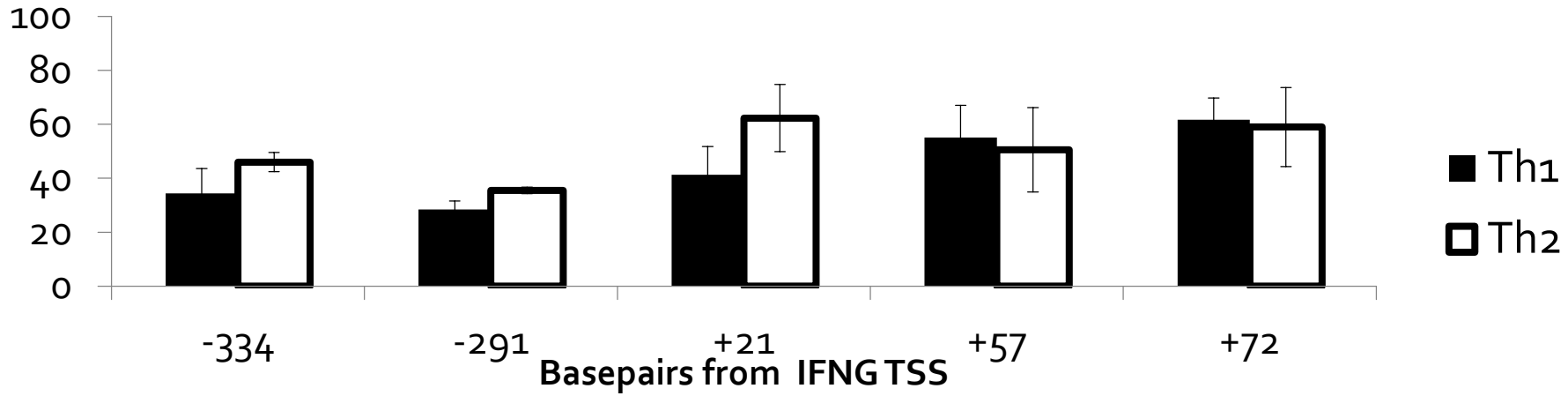


IL-4 and IFNγ Gene Expression after Th1-Th2 Skewing of Isolated CD4+ T-cells (n=5 cows)

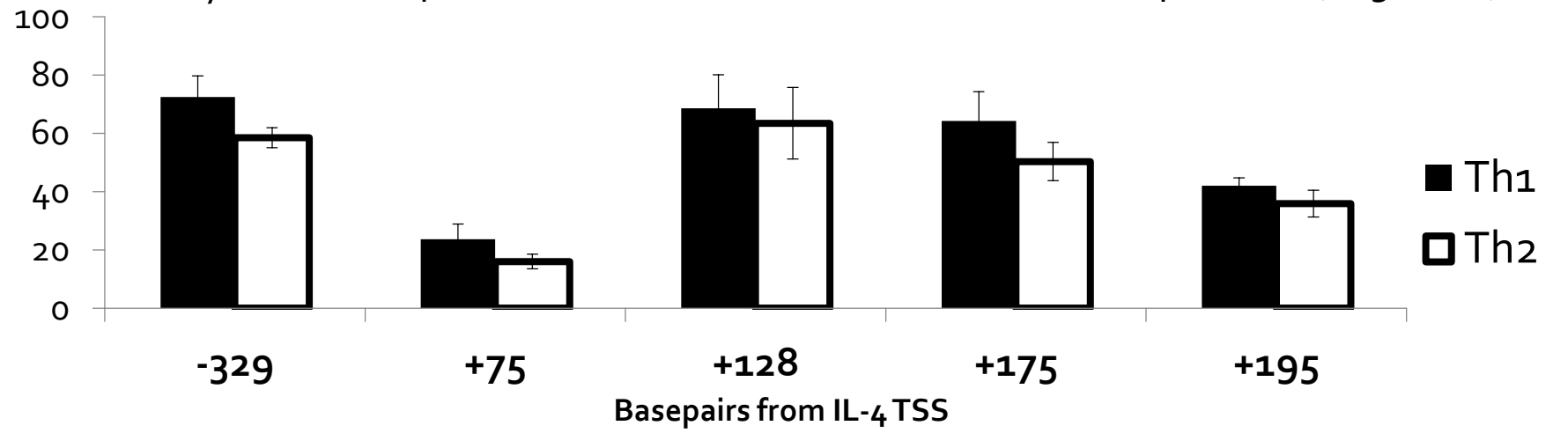


DNA Methylationof Bovine Type 1 and Type 2 Skewed CD4+ T-cells

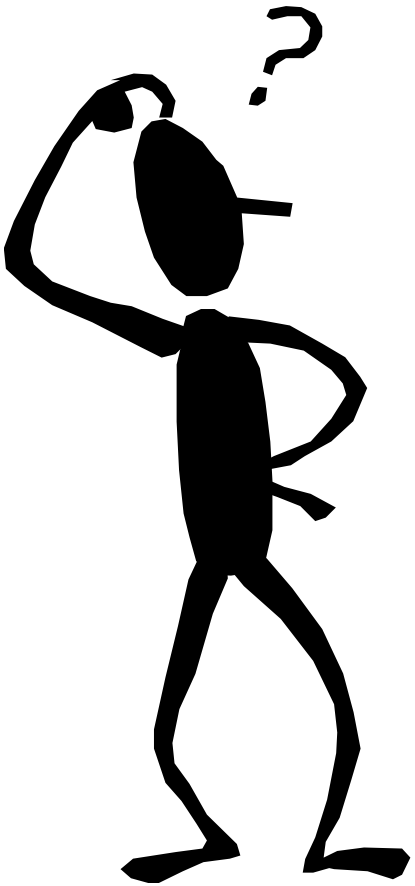
DNA Methylation of IFNG Promoter of Th1-Th2 Skewed Bovine CD4+T-cells(n=5 cows)

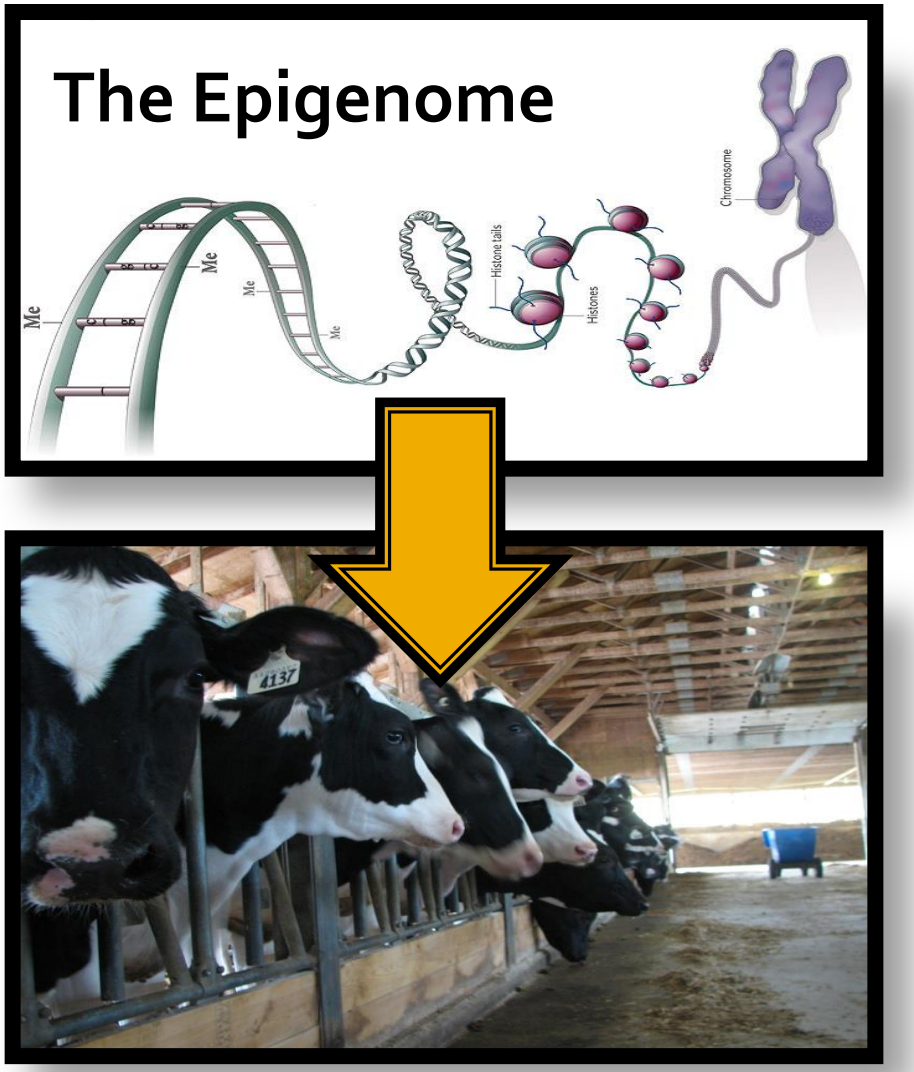


DNA Methylation of IL4 Promoter of Th1-Th2 Skewed Bovine CD4+T-cells (n=5 cows)

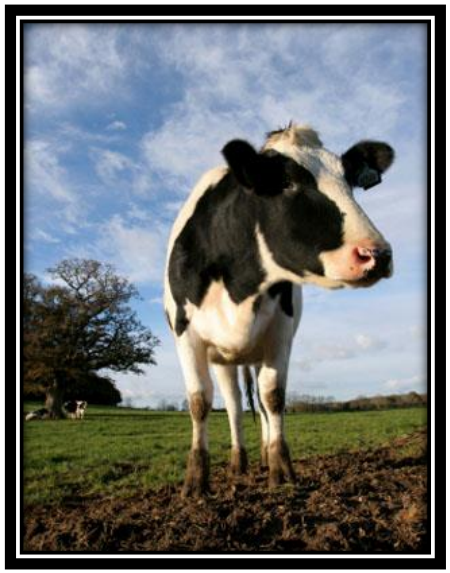


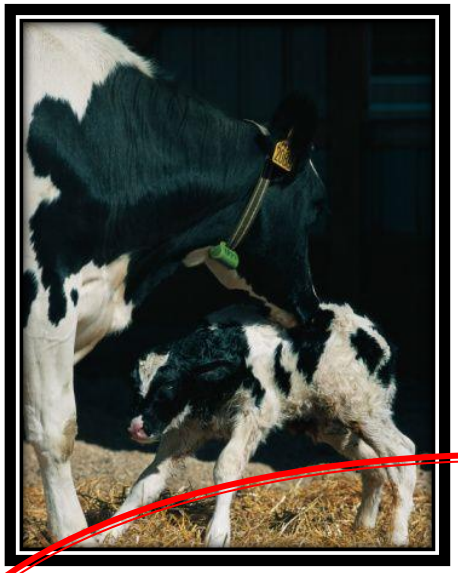
How can we apply this on farm?





Parturition Effects on Immune Response





Pregnancy | Parturition | Postpartum

AMIR | **CMIR** | **AMIR/CMIR?**

Peripartum Period

- Peripartum period reflects a period of stress in dairy cows
- Higher occurrences of disease after calving
  - e.g. Mastitis, ketosis, metritis
- Evidence of suboptimal immune response
  - (Mallard et.al.,1998)
- Characteristic shifts in Th1- Th2 responses
  - (Shafer-Weaver et.al.,1999)

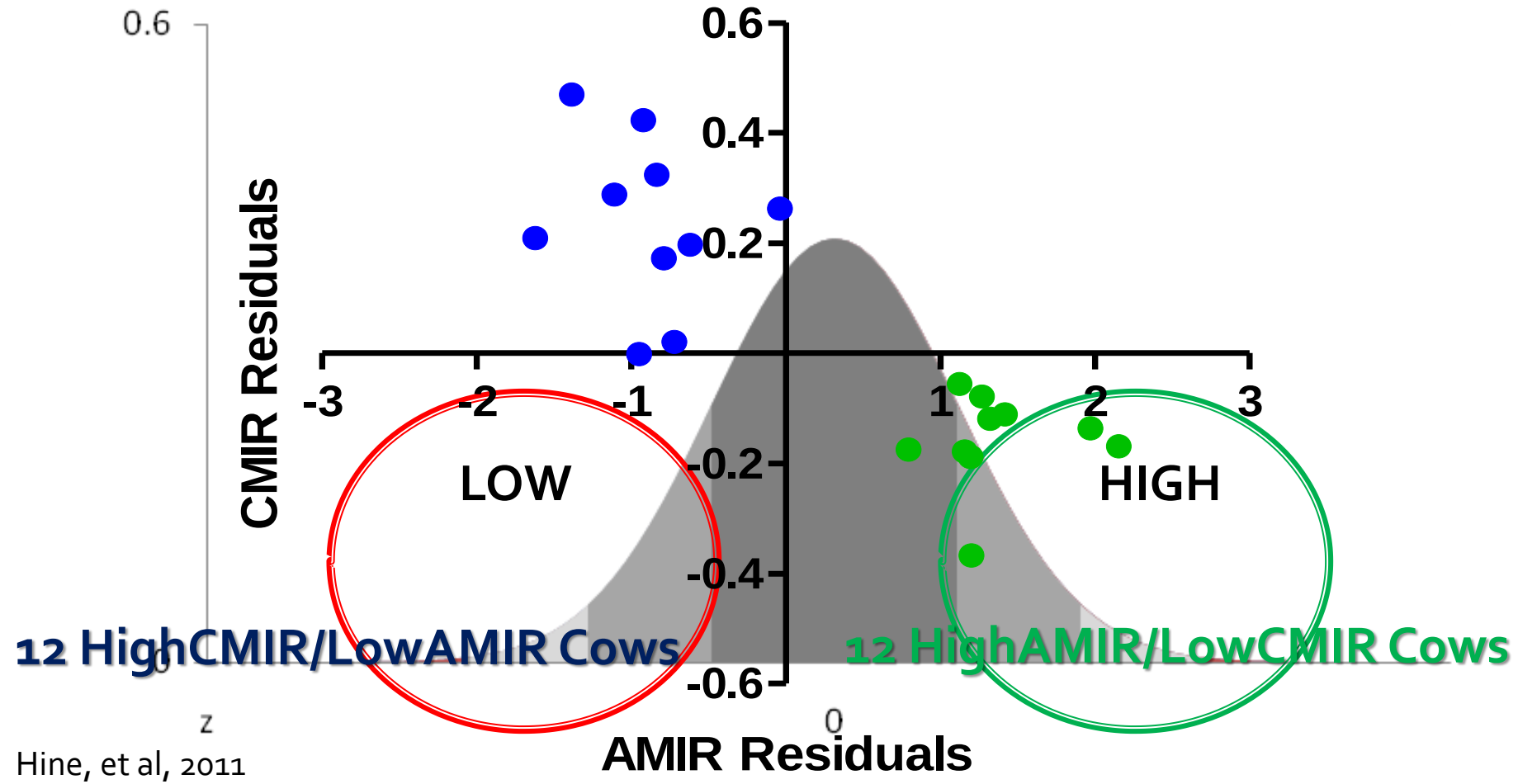
High Immune Response™

Cell-Mediated Immune Response (CMIR)

Antibody- Mediated Immune Response (AMIR)

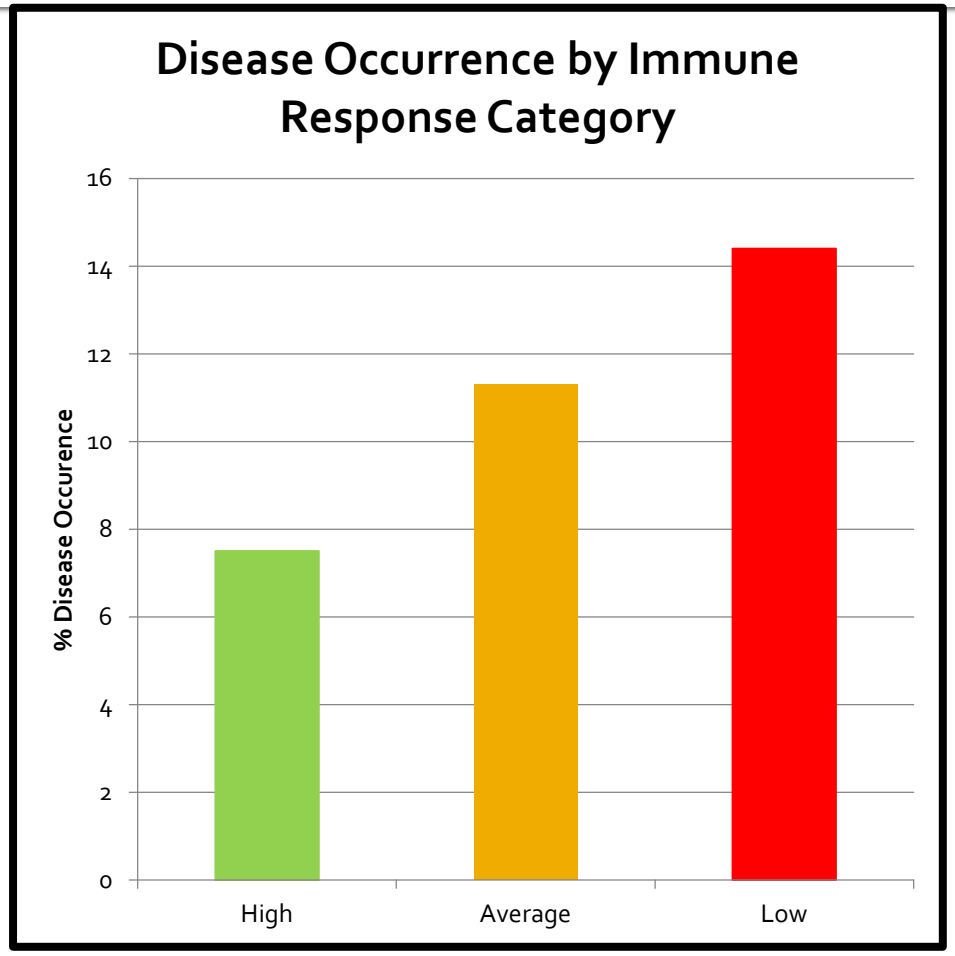
Immune Response Traits

- High and low groups are ±2/-1 standard deviation from mean



Hine, et al, 2011

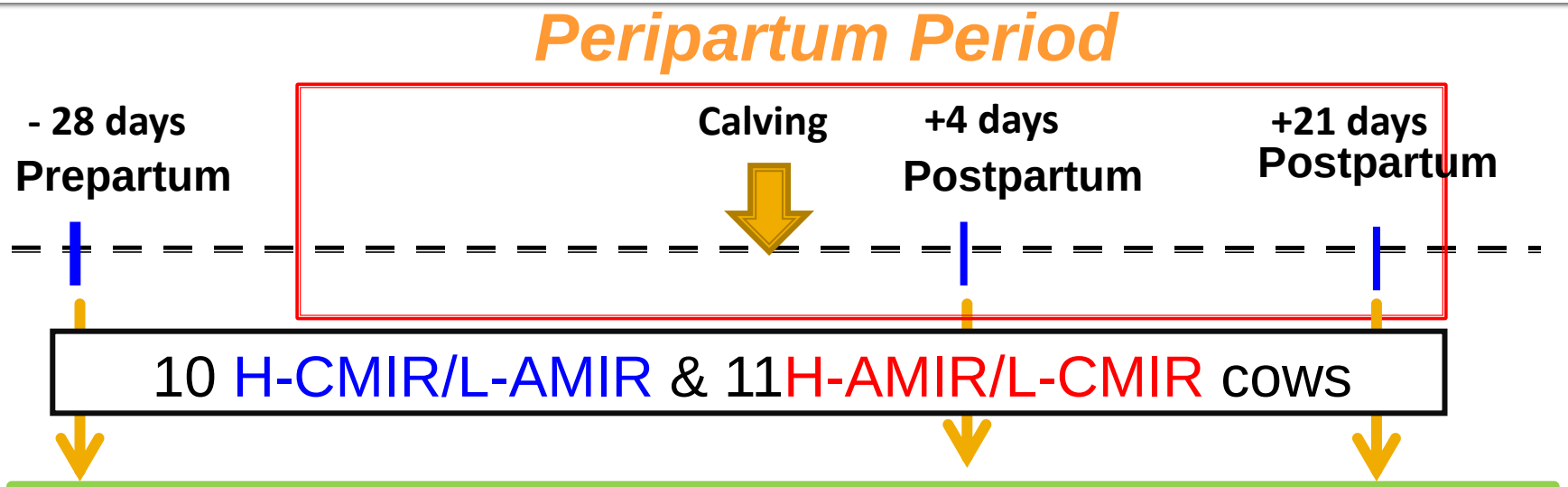
High Immune Response™



Immunity+™
Disease Resistant Genetics

Wagter LC. 2000 J. Dairy Sci. 83(3):488; 2008 DeLapaz, J. MSc Thesis University of Florida; Thompson-Crispi K. 2012. JDS 95(7):3888; Thompson-Crispi K. 2013. Clin Vacc Immuno. 20(1):106.

Methods



Whole Blood as a source of

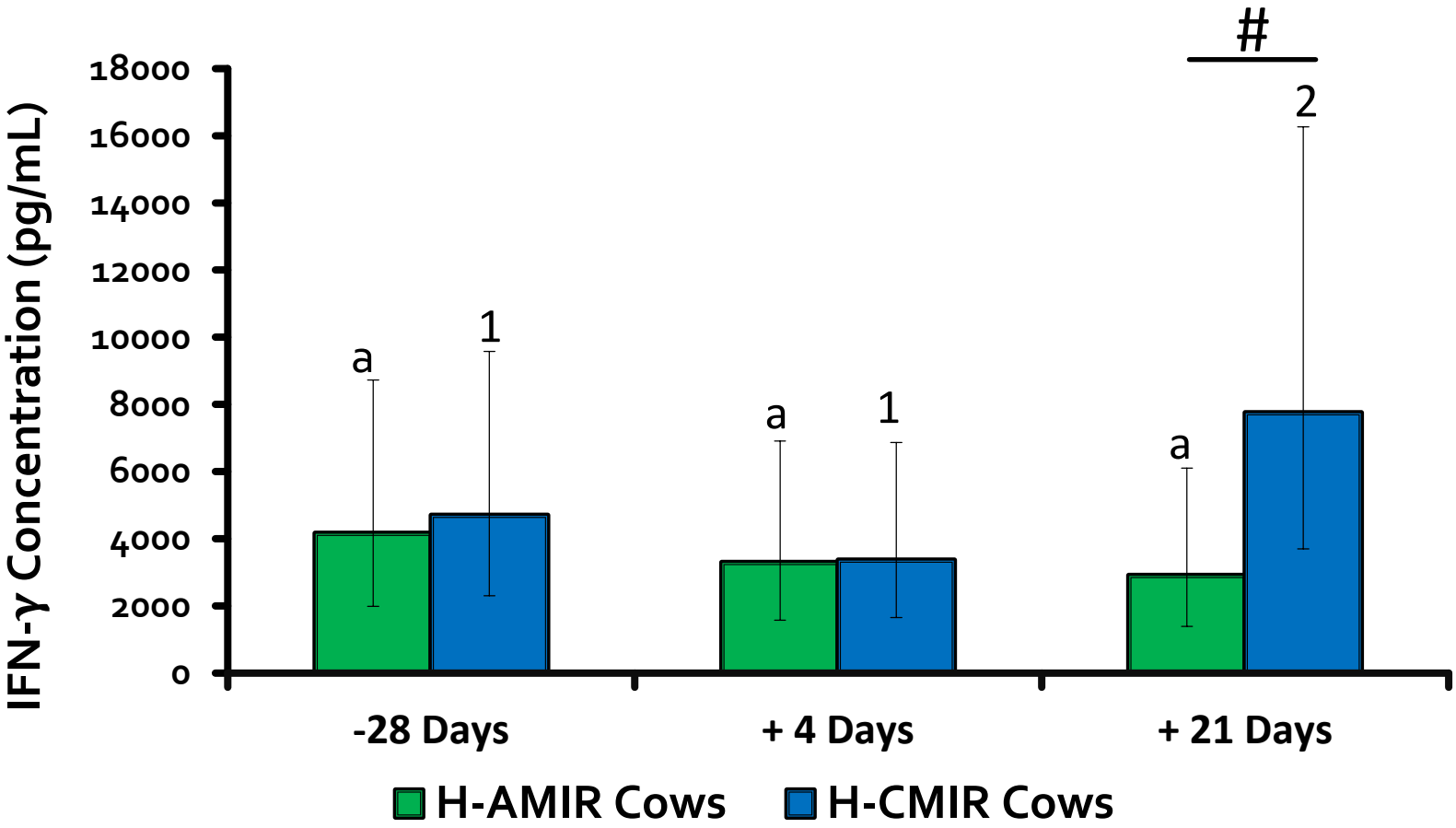
- CD_4+ T lymphocytes (Density gradient and MACS)
- Stimulated with T-cell mitogen (ConA)
- Cell culture supernatant for measuring cytokines (ELISA)
- DNA for methylation profiling (Bisulfite Conversion Assay)

- PROC MIXED in SAS9.2.1.; log (concentration) to normalize the data.

$$Y = \mu + GROUP + TIME + CYTOKINE + GROUP*TIME*CYTOKINE + PARITY + \varepsilon$$

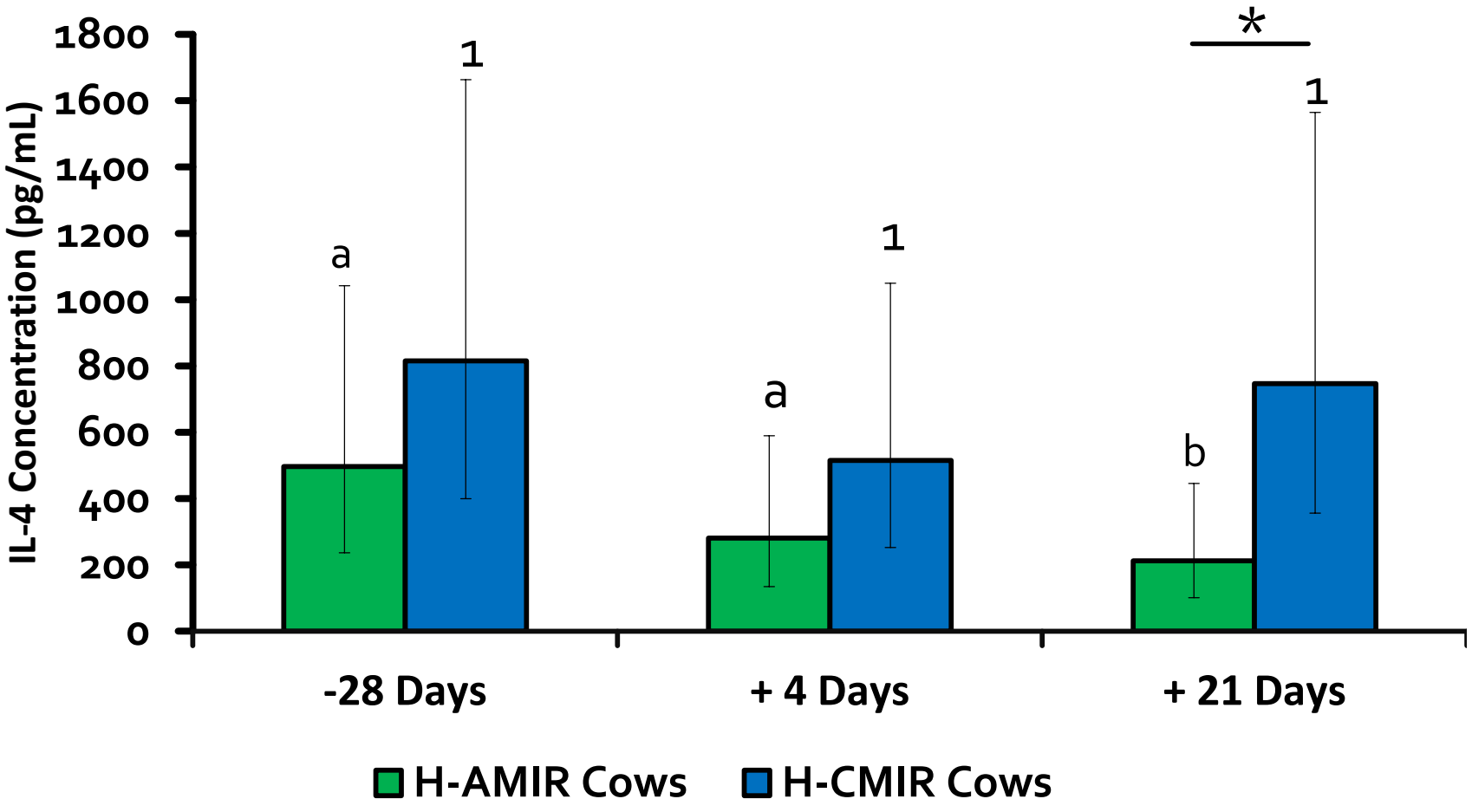
GROUP= HiAMIR/LoCMIR or HiCMIR/LoAMIR; Time= -28, +4, +28 Days; Cytokine= IFN, IL4, IL17A; Parity=1st & 2nd

H-CMIR Produce More Cytokines



# p<0.10

H-CMIR Produce More Cytokines



* p<0.05

What would we expect to see?

More cytokine after stimulation of T-helper cells isolated from H-CMIR

=

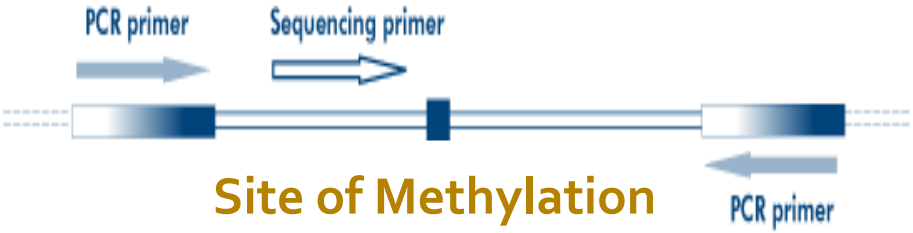
Less methylation for both cytokines in H-CMIR cows

Bisulfite Pyrosequencing

Magnetic bead Isolated T-Helper Cells

Extract DNA

Treat with Bisulfite



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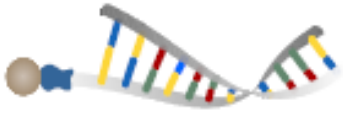

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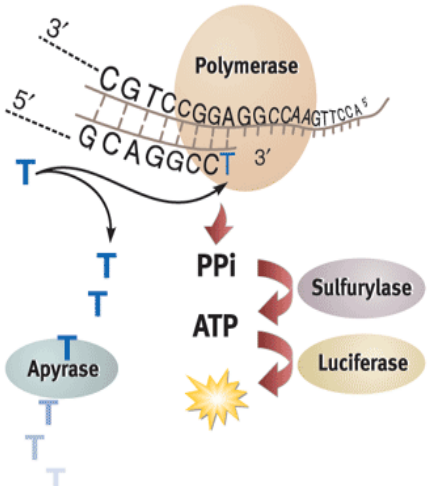
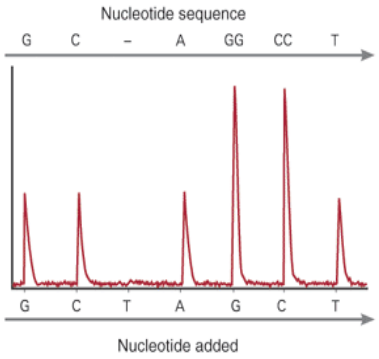
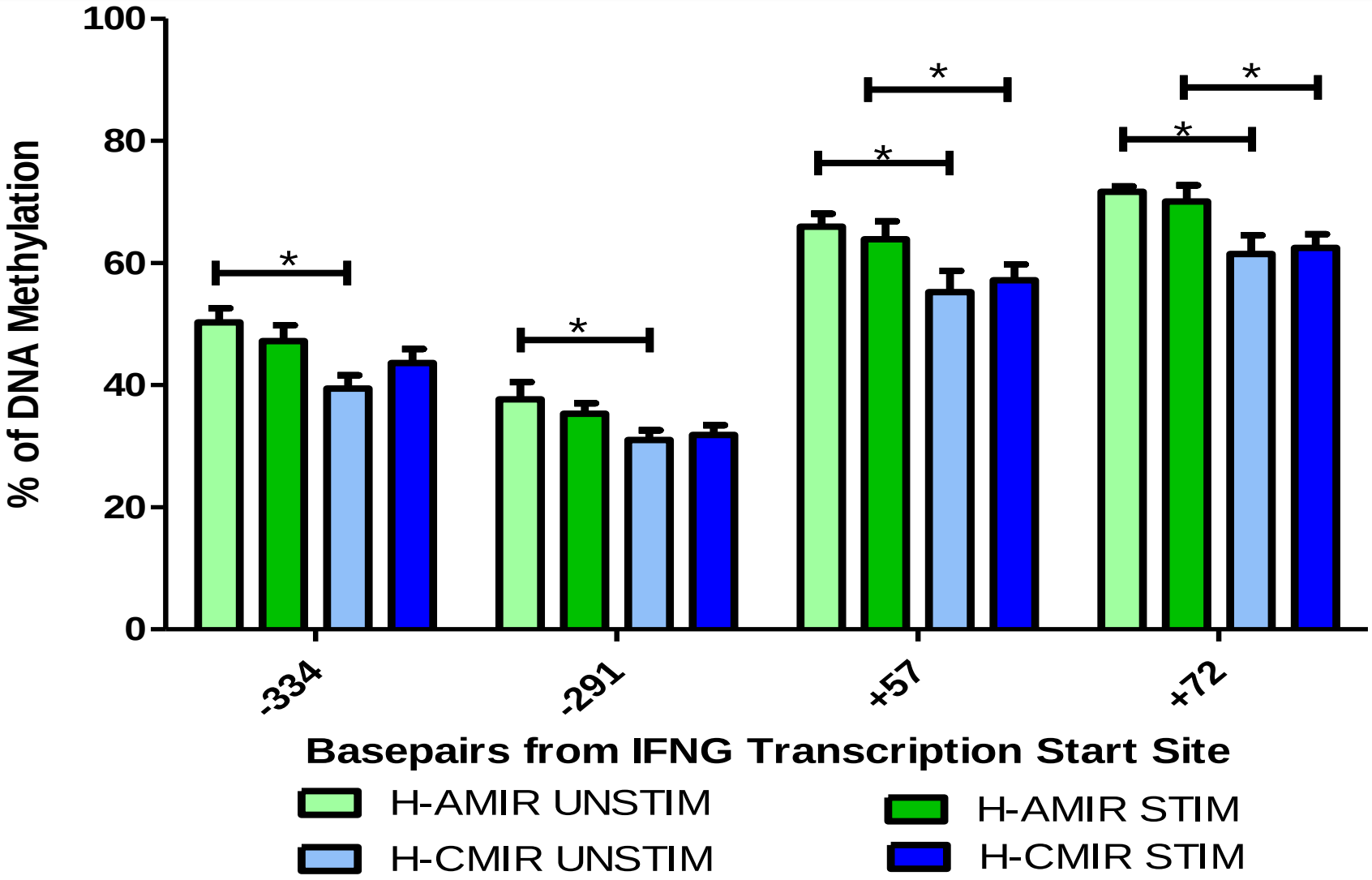
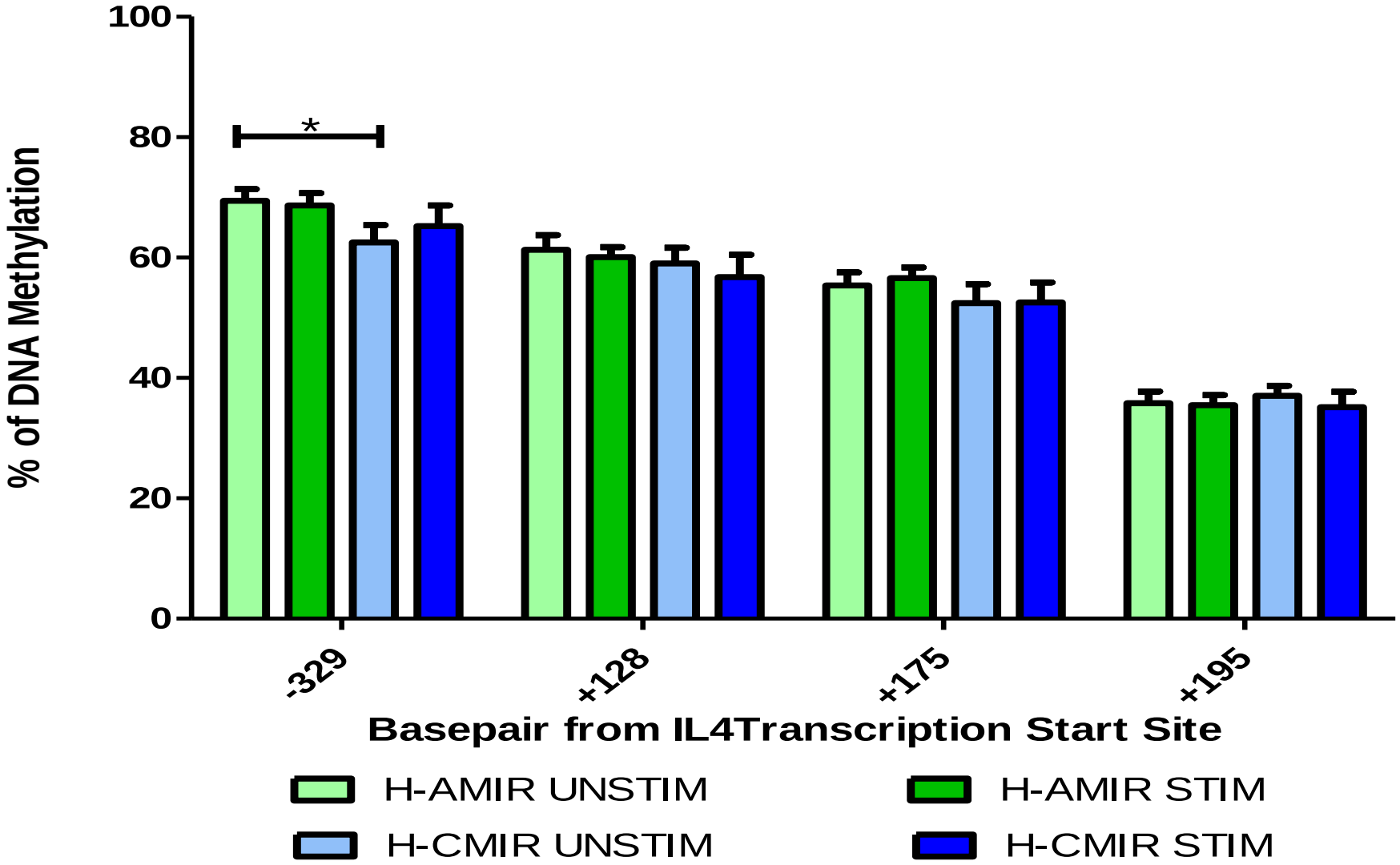




DNA Methylation of IFNG Promoter from Isolated CD4+ T-cells of H-AMIR (n=11) & H-CMIR (n=10) Dairy Cows



DNA Methylation of IL4 Promoter from Isolated CD4+ T-cells of H-AMIR (n=11) & H-CMIR (n=10) Dairy Cows



Conclusion

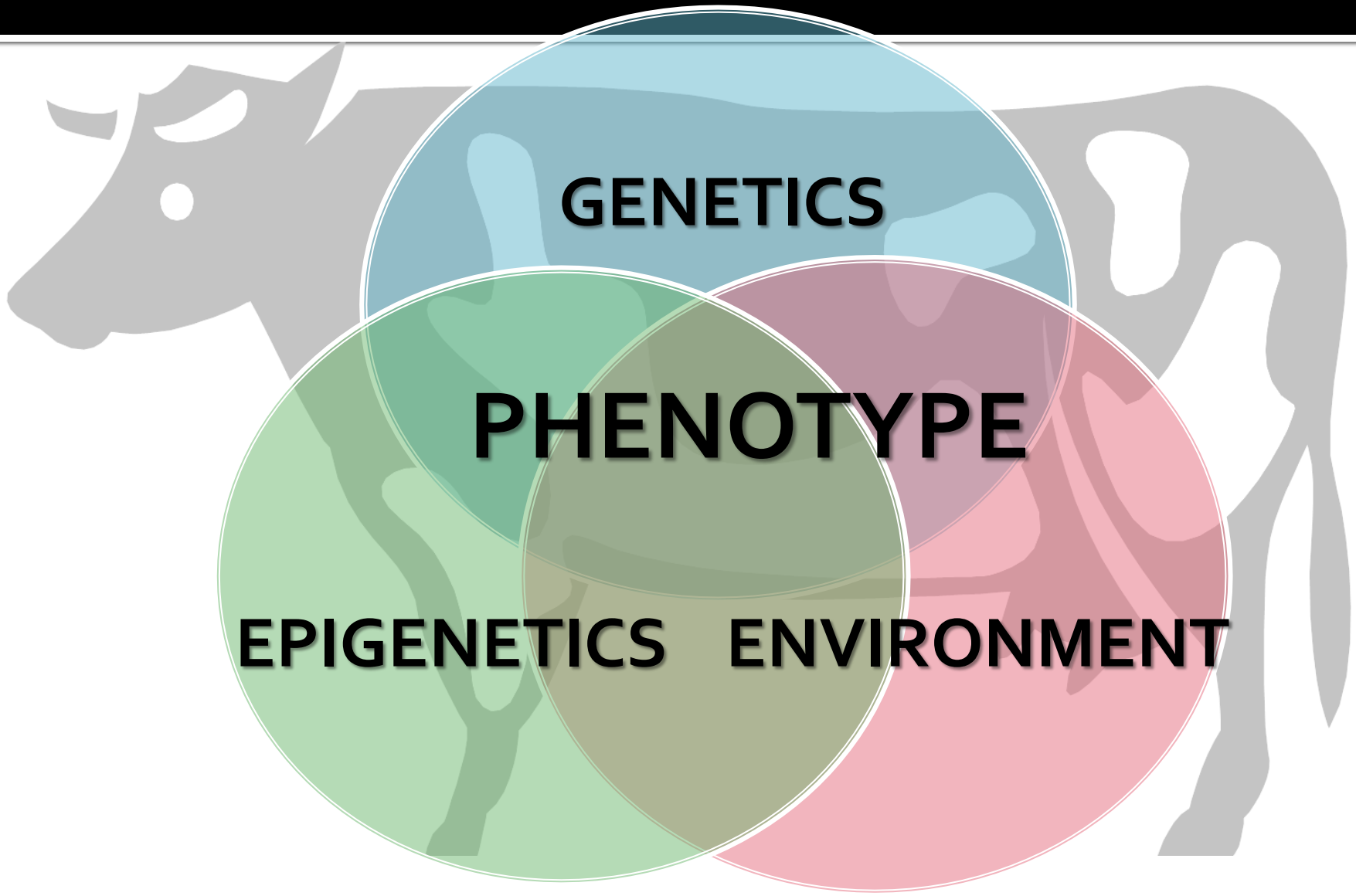
H-AMIR cows **less** cytokine production

↑ DNA methylation

H-CMIR cows **more** cytokine production

↓ DNA methylation

What you do today can effect the generations of tomorrow



Thank you

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• Advisor: Dr. Bonnie Mallard
  •Dr. Shayan Sharif
  •Dr. Niel Karrow

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•Funding: NSERC Discovery

National Milk Federation PhD Paper Competition: Production Division

•American Dairy Science Association
•Novus International