Influence of lactation during pregnancy on epigenetic regulation of genes in dairy cattle

Alex Bach and Anna Aris

IRTA
INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIAS

icrea
Institució Catalana de Recerca i Estudis Avançats

Introduction

- For many years, genetic progress in dairy cattle has taken place by selecting for the “right” traits

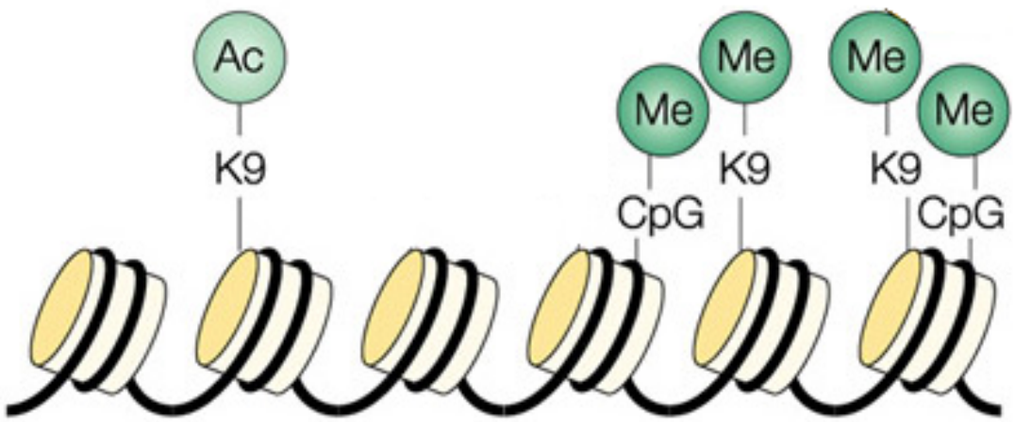
- With genomics, now genetic selection is also based on the existence or absence of specific genes (regardless of their expression)

- Fetal programing refers to the impact that the uterine environment exerts on the offspring through differential expression of genes

Introduction

- Mechanisms behind fetal programming and metabolic imprinting are mostly epigenetic

- DNA methylation, the addition of a methyl group to a cytosine base followed by a guanine (CpG), is the most widely studied epigenetic modification

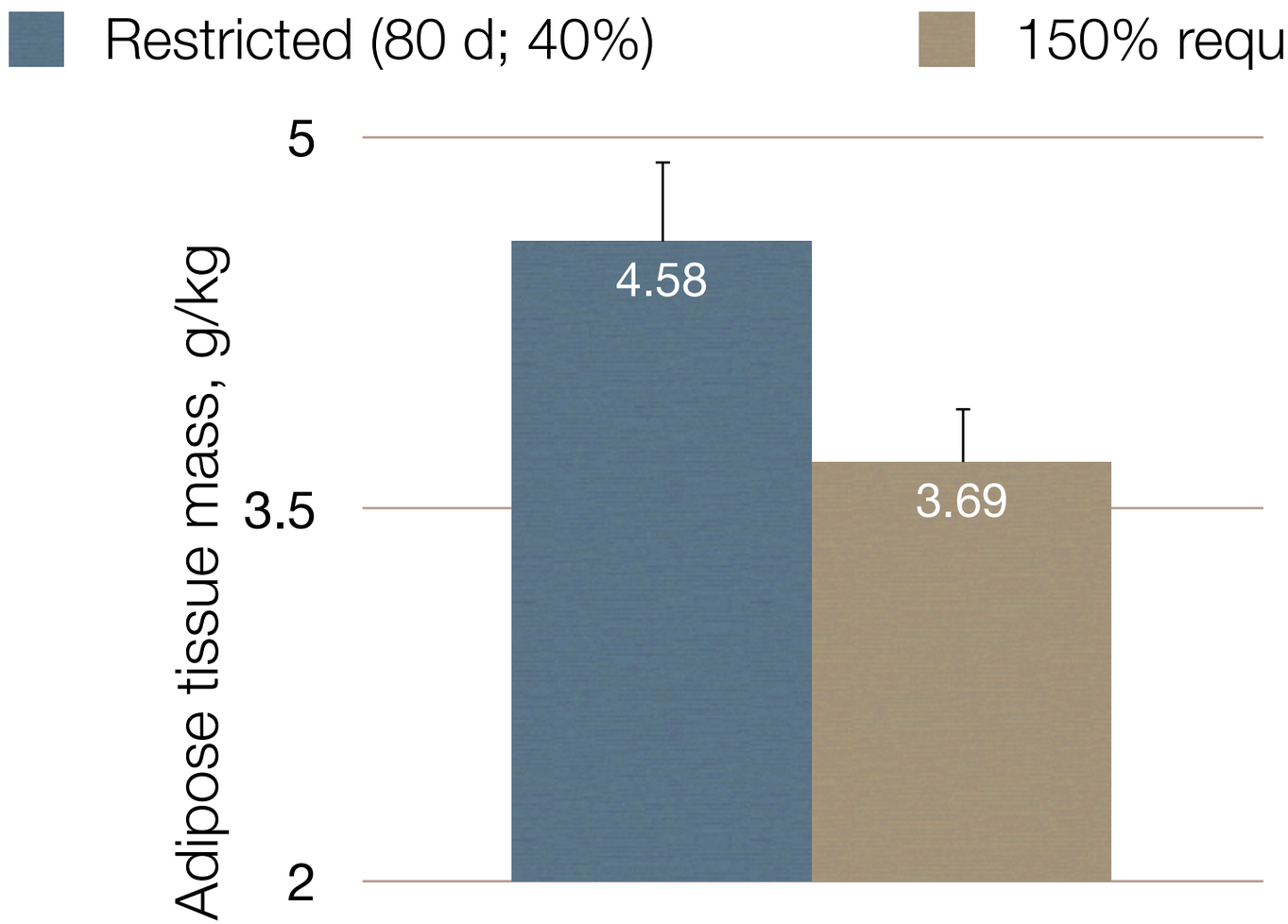


Adapted from Andrew et al., 2004

Encyclopedia Britannica, Inc., 2006

Introduction

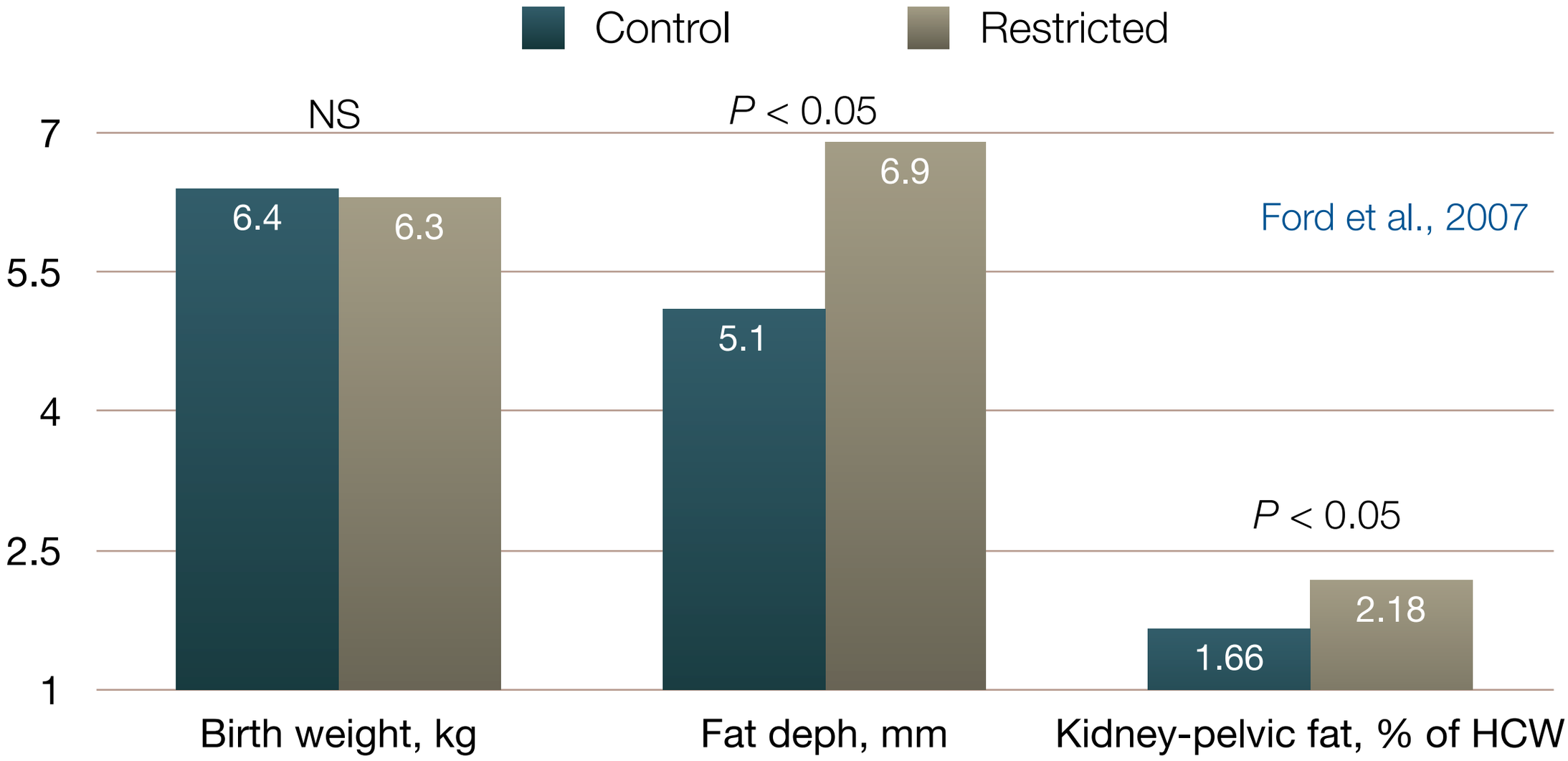
- In sheep, caruncular growth and metabolic activity is affected by maternal nutrition (Hruska et al., 1991)
- A severe or a modest maternal under-nutrition early in pregnancy increased adiposity of the offspring



Bispham et al., 2003

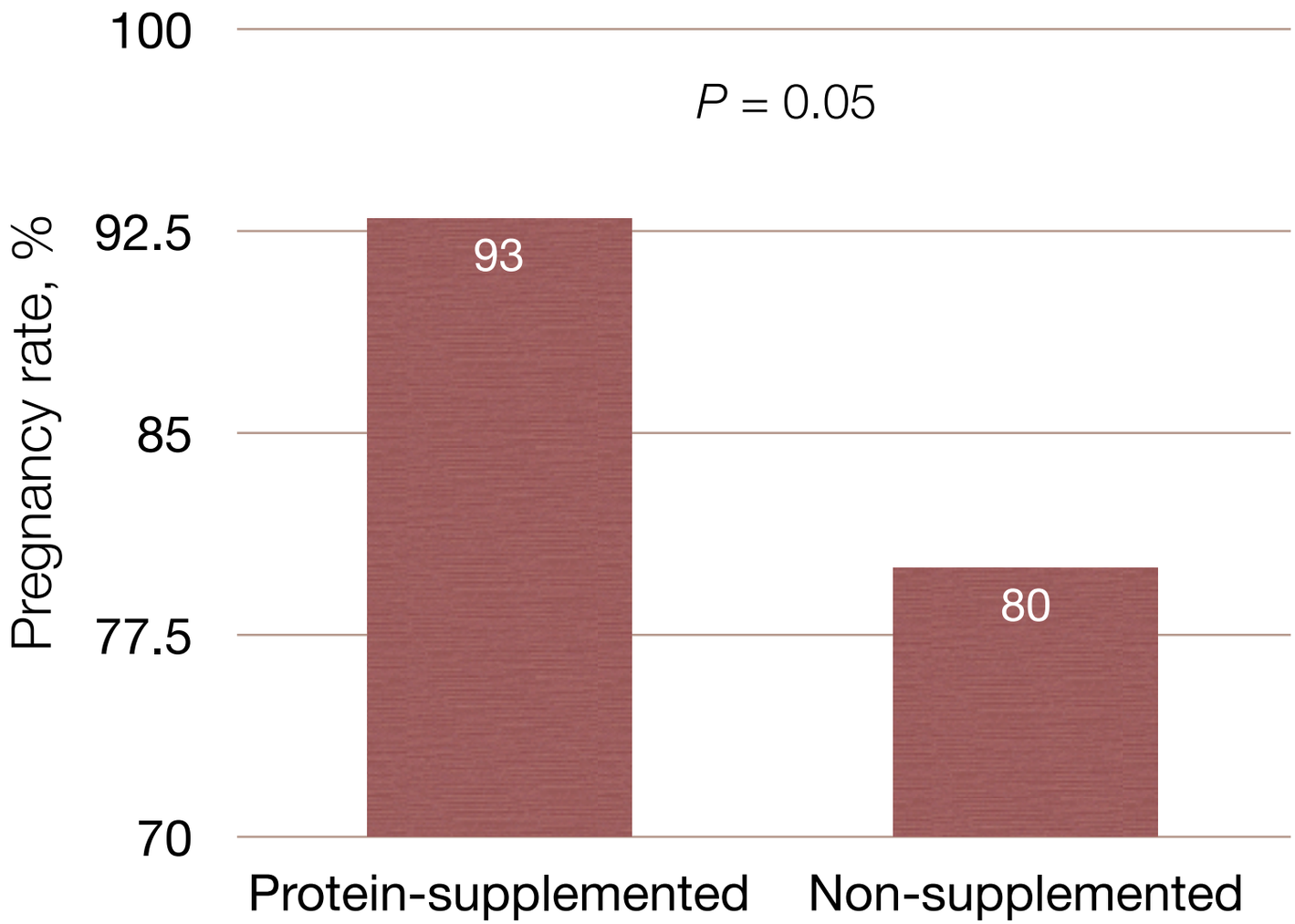
Introduction

- Undernutrition of beef cattle compromises placental angiogenesis, cotyledon weight, and fetal development (Vonnahme et al., 2007; Long et al., 2009; Sullivan et al., 2009)



Introduction

- The mother's metabolic status can also affect reproductive function of the offspring
- A protein deficiency (65% of recommendations) in primiparous beef cows during last 100 d of pregnancy delayed age at puberty of the progeny (Corah et al., 1975)

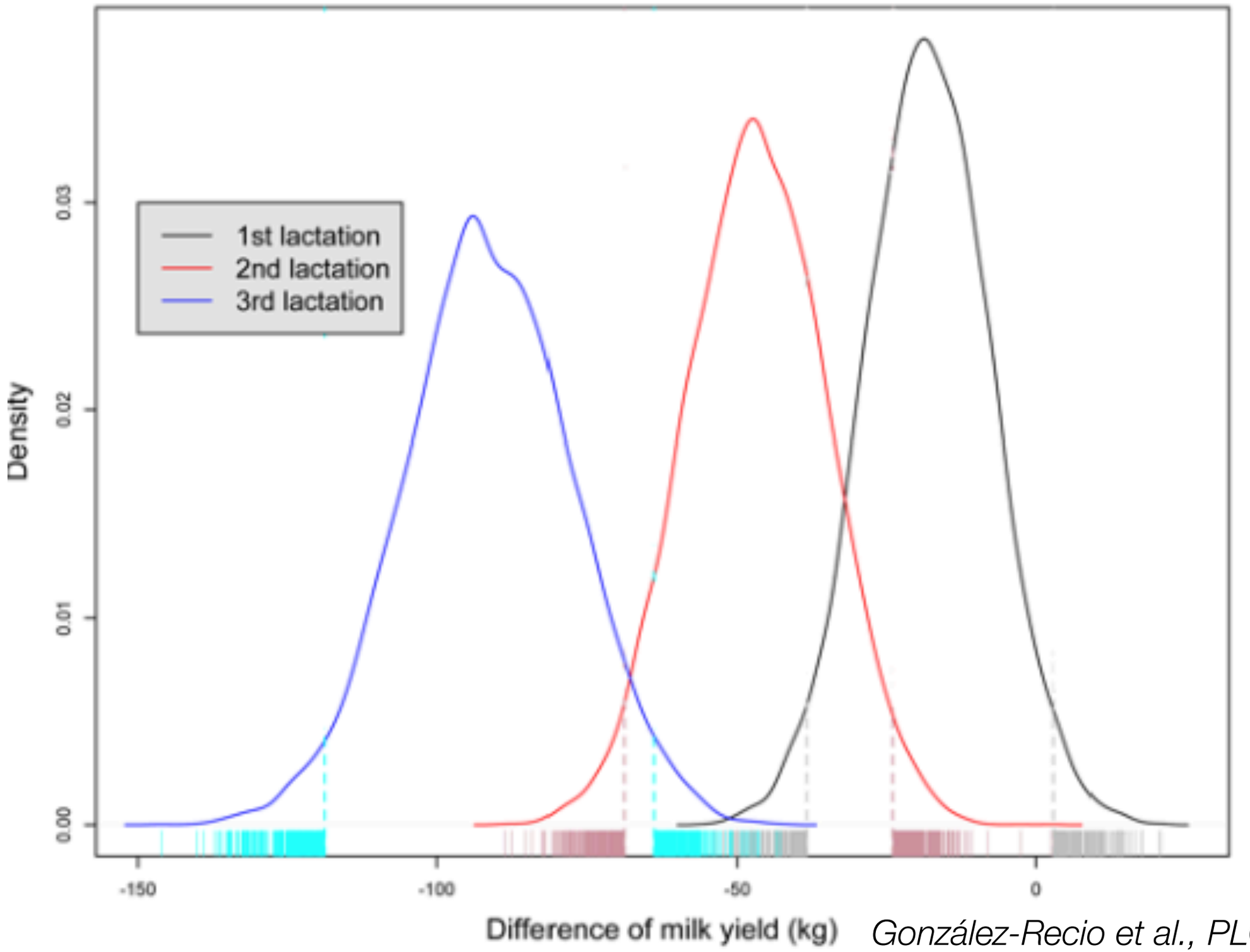


Martin et al., 2007

Introduction

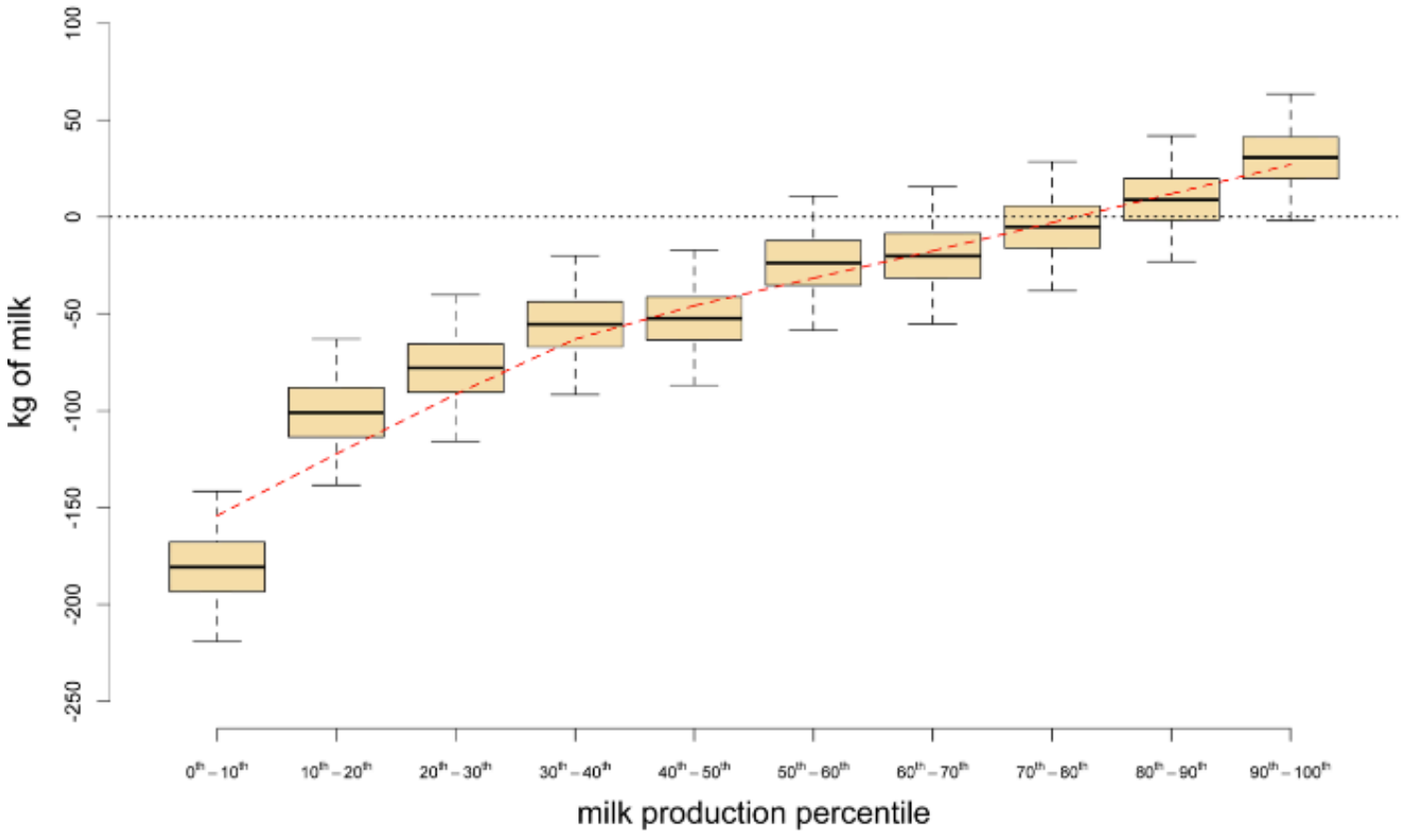
- Under optimal breeding programs, newborn calves are the animals within a herd with the greatest potential for profitability:
  - Have the greatest genetic potential
  - Give birth to cows that:
    - Have greater longevity
      and
    - Produce more milk

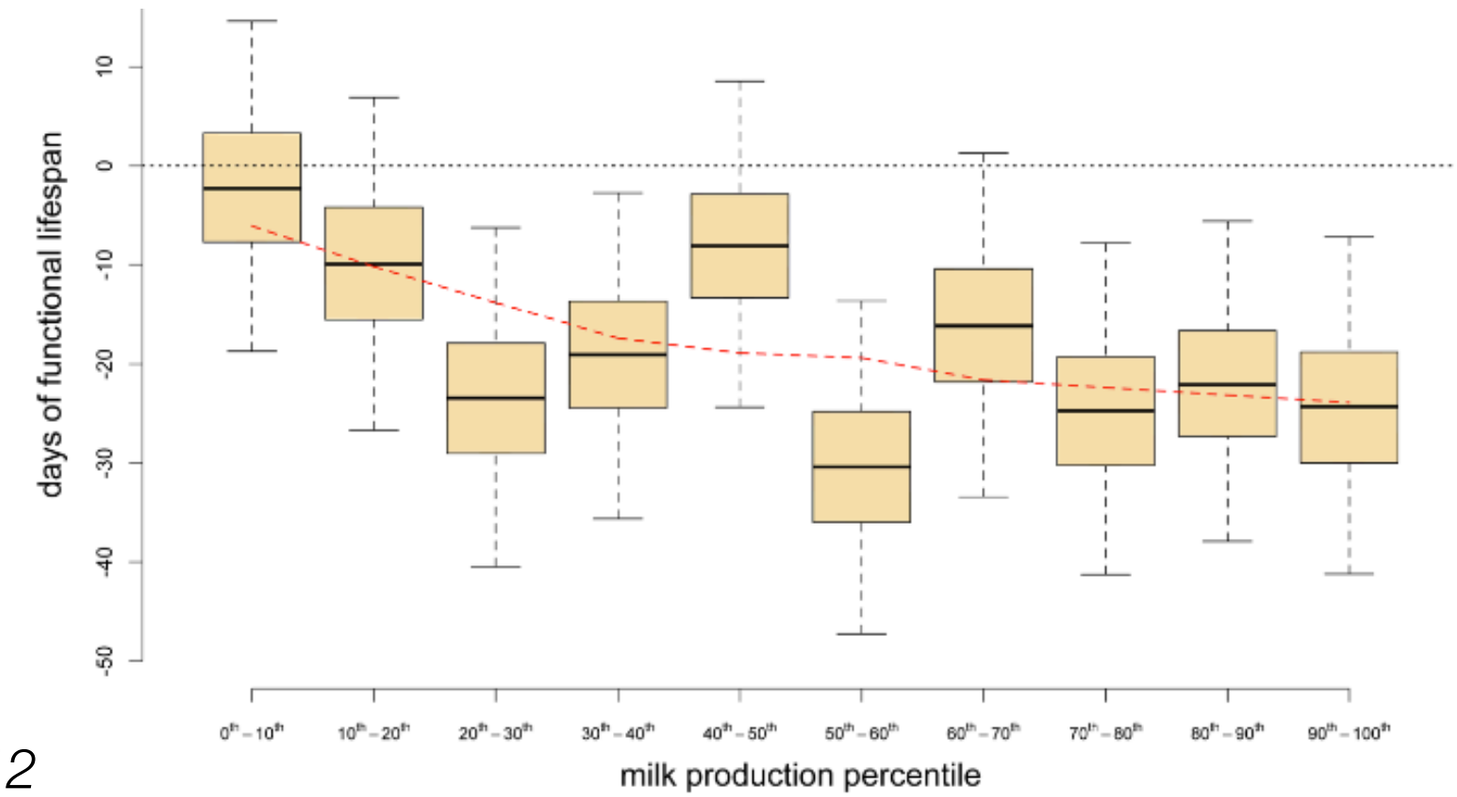
Introduction



González-Recio et al., PLOS One 2012

Introduction

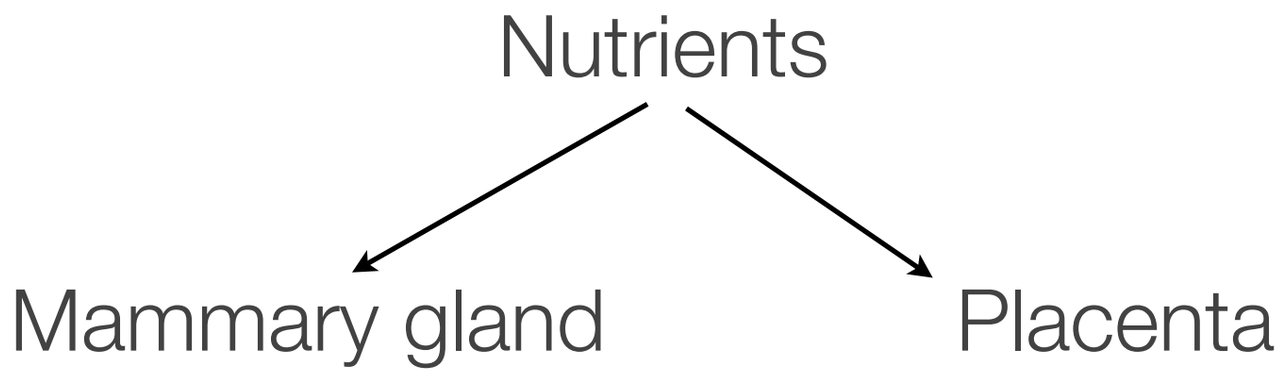




González-Recio et al., PLOS One 2012

Introduction

- Coexistence and dispute between placental and fetal development and milk production



- Banos et al. (2007): no relationship

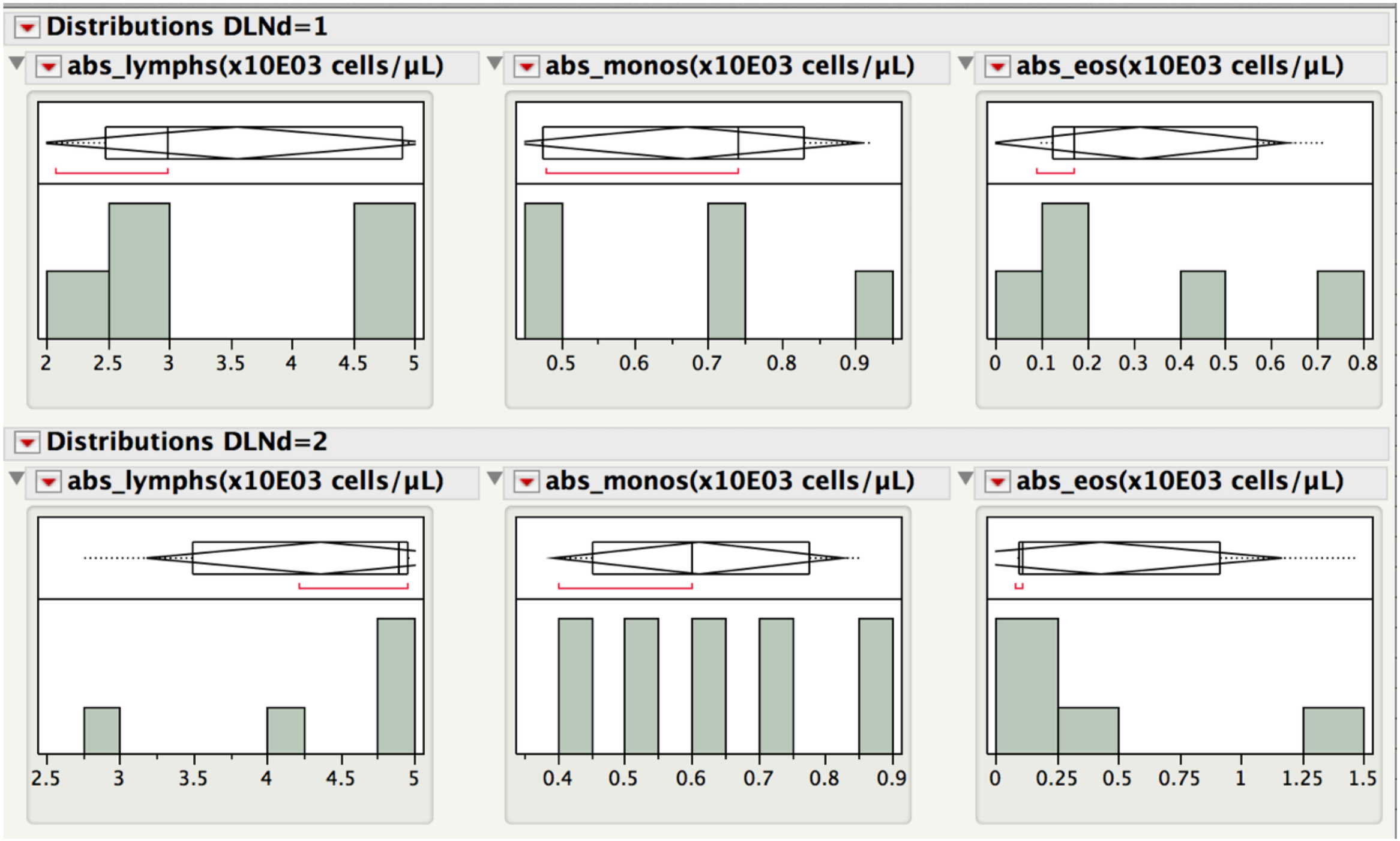
- Berry et al. (2008) maternal milk yield negatively correlated with progeny milk yield in 1st and 3rd lactations

- Past and current nutritional models have focused on maximizing performance and minimizing metabolic disorders
  - Pregnancy only *exists* the last 3 months pre-calving

Materials and Methods

- Hypothesis: lactation during pregnancy will influence the epigenome of the offspring
- Ten coetaneous half-sisters (same sire) female Holstein calves (42.9±6.87 kg of BW) either born to a **heifer** (*n*=5) or born to a **multiparous** cow (*n*=5) were bled at the age of 27±5 d by venipuncture of the jugular vein
- Blood samples were processed to make sure no differences in the hematogram were present

Materials and Methods



4.3

0.61

0.43

Materials and Methods

- After confirming the absence of differences, DNA was extracted and processed to run a DNA methylation micro-array with 362,212 CpG bovine probes (Agilent)

- Samples were immunoprecipitated using a 5-methylcytosine antibody

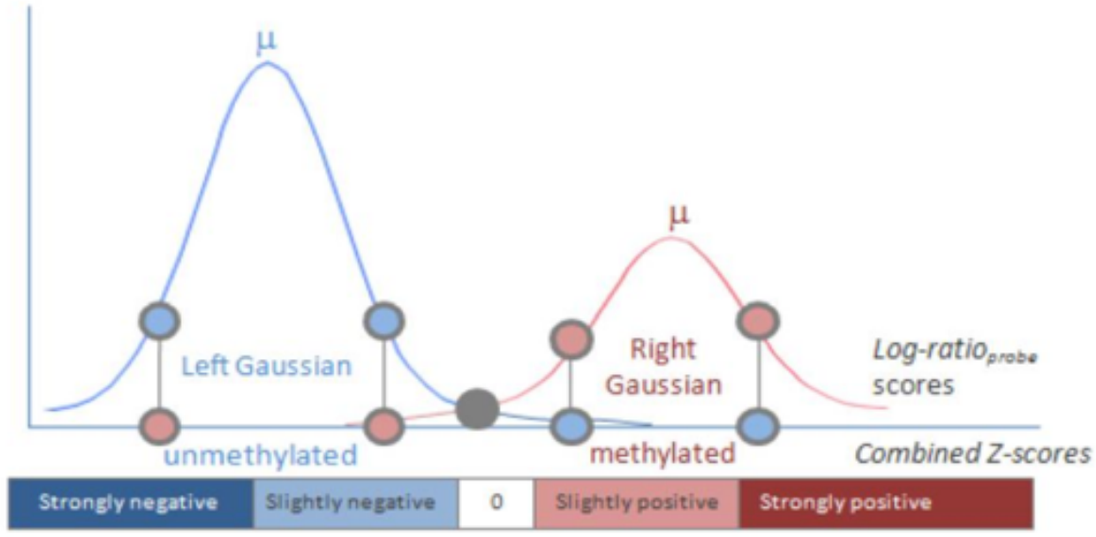
- A labeled immunoprecipitated DNA – Cy5 and reference DNA – Cy3 were combined for the hybridization, denatured at 95 °C for 3 min and incubated at 37 °C for 30 min

- The samples were hybridized and scanned at 3 microns using an Agilent Scanner

Materials and Methods

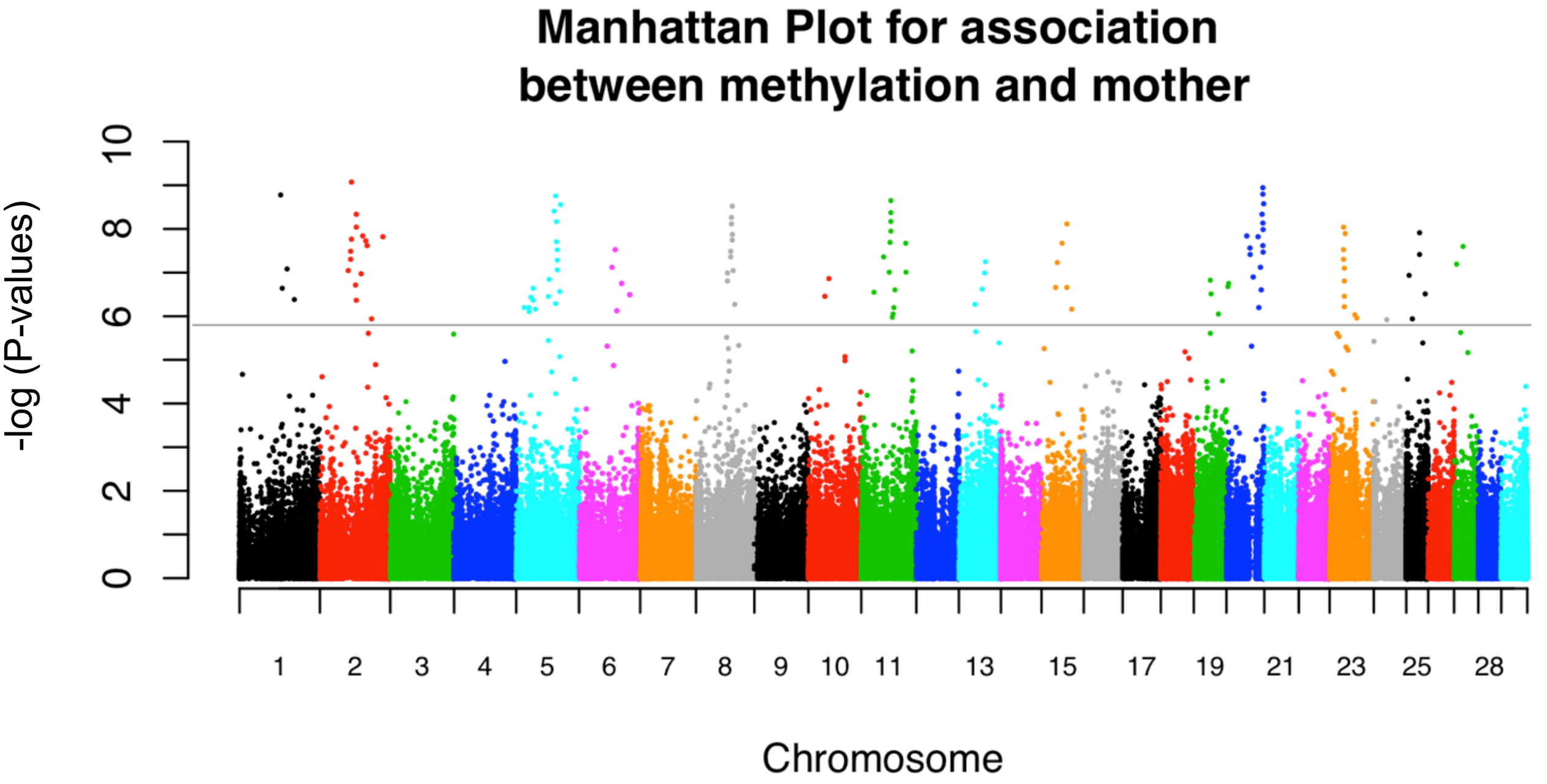
- The data were normalized using linear normalization.

- The methylation signal was processed using the Batman algorithm

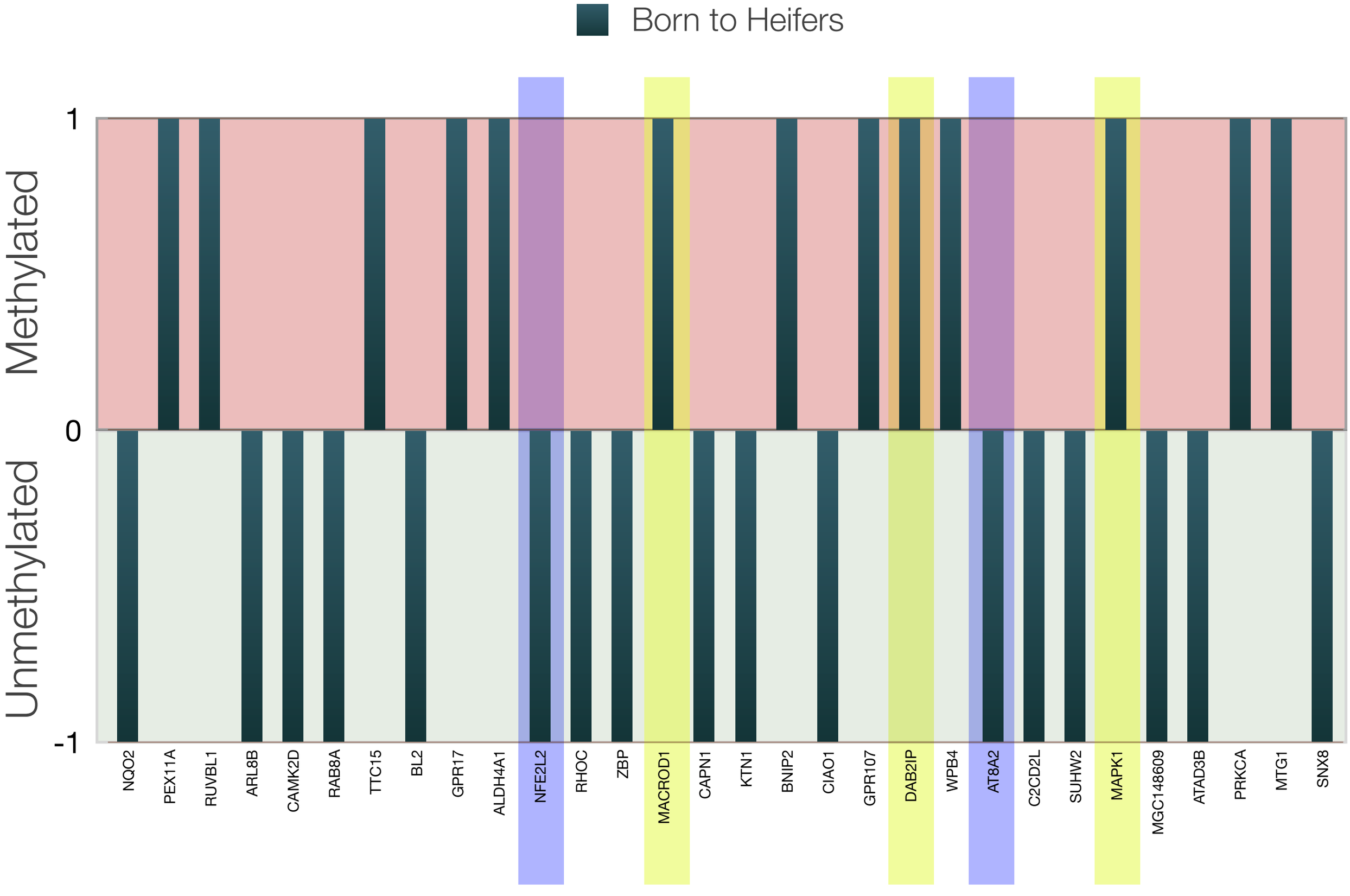


- Then, the data were processed using MethLab with a Beta model (log ratio of methylated to unmethylated signal) with chip as a random effect and parity of the dam (heifer or cow) as a fixed effect

Results


Manhattan Plot for association between methylation and mother
-log (P-values)
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
11
13
15
17
19
21
23
25
28
Chromosome

Results


Born to Heifers
1
0
-1
Methylated
Unmethylated
NQO2
PEX11A
RUVBL1
ARL8B
CAMK2D
RAB8A
TTC15
BL2
GPR17
ALDH4A1
NFE2L2
RHOC
ZBP
MACROD1
CAPN1
KTN1
BNIP2
CIAO1
GPR107
DAB2IP
WPB4
AT8A2
C2CD2L
SUHW2
MAPK1
MGC148609
ATAD3B
PRKCA
MTG1
SNX8

Results

- All 5 calves born to heifers had NFE2L2 and ATP8A2 unmethylated, whereas all 5 calves born to multiparous cows had them methylated (reduced expression)

- These are important genes participating in the generation of energy and controlling oxidative stress (Chamala et al., 2006; Gold et al., 2012)

Results

- All 5 calves born to heifers had DAB2IP and MAPK1 methylated, whereas all 5 calves born to multiparous cows had them unmethylated

- DAB2IP (Disabled homolog 2-interactin protein) fosters the activity of MAPK1

- Hyperexpression of MAPK1 (mitogen activated protein kinase 1) has been associated with impaired fertility (Gratao et al., 2007)

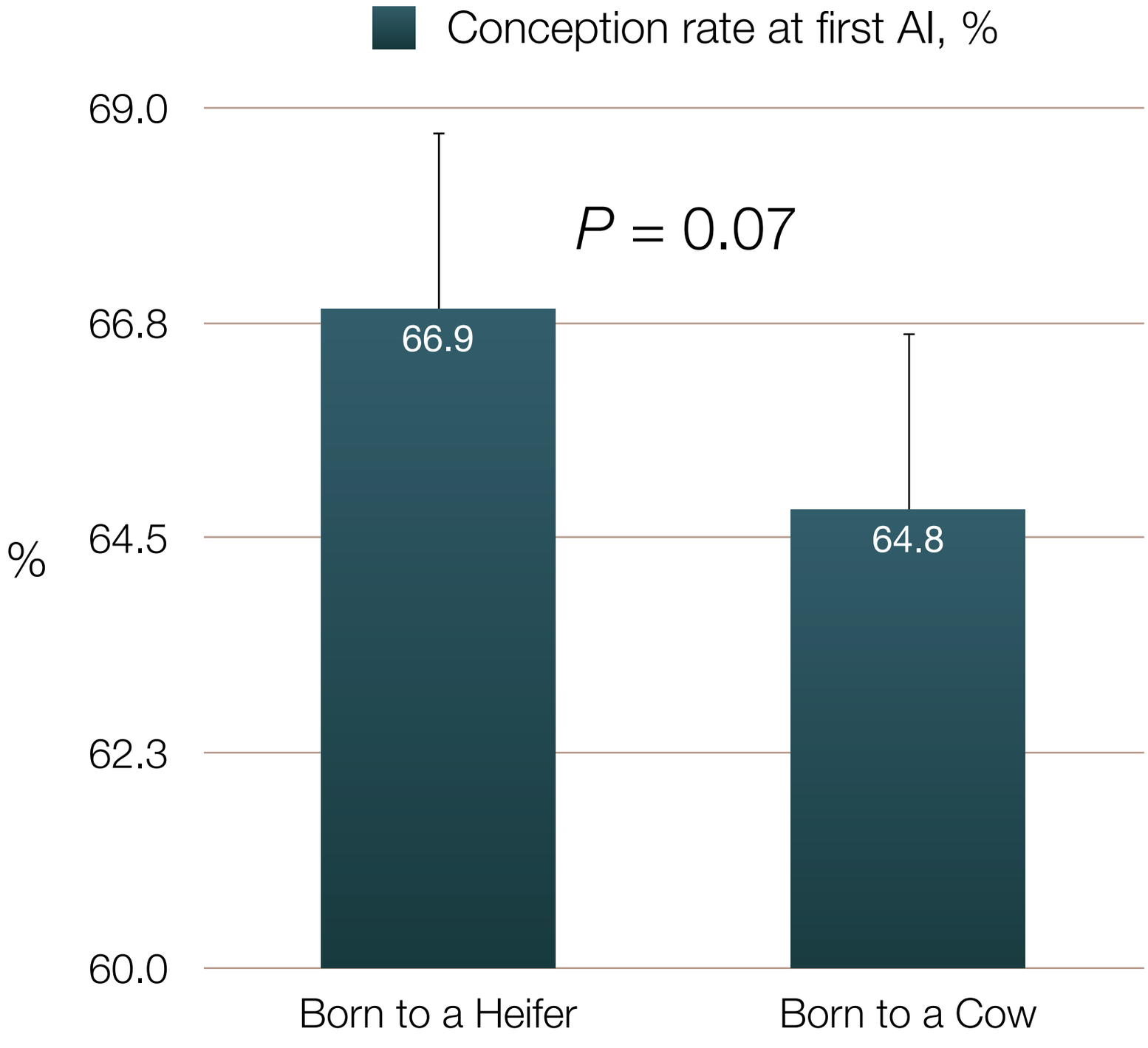
Results

- A dataset from 9,786 heifers raised and fed under the same conditions was used to evaluate potential differences in conception rate at first breeding depending on the parity of the dams

- Potential differences were assessed using a mixed-effects logistic regression model that accounted for the random effect of year of birth, season of breeding, and herd of origin, plus the fixed effects of parity of the dam and age at first breeding and their two-way interaction

Results

n=9,786 (3,861 born to heifers; 5,925 born to cows)



Conclusions

- The competition of nutrients between the placenta and the mammary gland results in long-term programming of the offspring

- The methylation status of more than 130 genes is greatly affected by the co-existence of lactation with gestation

- Some important genes participating in energy utilization are un-methylated in calves born to heifers

Conclusions

- Some relevant genes influencing reproductive performance are methylated, which should improve conception rates

- Empirical data support this observation

- Need to look for nutritional strategies during lactation of pregnant cows that would minimize the negative consequences on the metabolism and reproduction of the offspring

alex.bach@irta.cat

Thank you